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(54) Title: HOMOCONJUGATED IMMUNOGLOBULINS

(57) Abstract

Homoconjugated antibodies with high avidity for antigen have increased therapeutic activies and are utilized in pharmaceutical and diagnostic compositions. The homoconjugates, typically prepared from monoclonal antibodies of the IgG class which bind to the same antigenic determinant, are covalently linked by synthetic cross-linking. The homoconjugates are comprised of at least two immunoglobulin monomers so as to provide an IgG-like molecule which is tetravalent, hexavalent or more for the selected antigen. The homoconjugates are able to cross the placenta. Methods of treatment using these homoconjugates are also provided.

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HOMOCONJUGATED IMMUNOGLOBULINS

Related Application

The present application is a continuation-in-part of Ser. No. 07/575,725, filed August 31, 1990.

Background Of The Invention

Monoclonal antibodies offer great promise as exquisitely specific immunotherapeutic agents with potentially minimal side effects. Thus, monoclonal antibodies are being developed for a wide variety of applications, such as the treatment of tumors, infectious diseases and autoimmune disorders, regulation of the immune system, and others. Unfortunately, few monoclonal antibodies have the qualities that enable them to successfully make the transition from research and development to clinical regimen.

The therapeutic or diagnostic usefulness of a monoclonal antibody results from several factors, in addition to simply binding the desired antigen. The antibody must possess sufficient binding affinity, a measure of the inherent strength of the antibody binding to its corresponding epitope. It must also have a relatively high level of avidity, which reflects the overall stability of the antibody-antigen complex and is based on the valency of the antibody (and antigen) and the geometric arrangement of the interacting components. The affinity and avidity of different antibodies can vary widely.

Often the monoclonal antibody which is selected must be of an appropriate isotype or subclass thereof to efficiently initiate desired effector functions. These functions include fixation of complement, binding to effector macrophages or polymorphonuclear leukocytes, or other properties that may be required in a particular therapeutic application. Isotype also affects antibody bio-distribution, half-life, transplacental passage, and other characteristics.

In general, IgG antibodies would be preferred over IgM antibodies for most therapeutic uses. When compared to IgMs, IgGs typically possess longer in vivo half-lives, are able to cross the placenta to the fetus, and when formulated as a pharmaceutical composition may have a longer shelf life. IgG molecules are monomeric, however, and have only two antigen binding sites so the avidity is much lower than with a comparable IgM antibody, which is pentavalent and has ten antigen binding sites.

With conventional technology it is frequently very difficult to identify monoclonal antibodies having the desired antigen binding specificity, affinity, avidity and effector functions. Recombinant DNA techniques have been developed to avoid the unpredictable and labor intensive method of simply screening large numbers of antibody-producing fused or transformed cells. Genes encoding the antigen binding variable (or hypervariable) regions of an antibody having a desired binding specificity have been cloned next to genes encoding antibody constant regions which mediate desired effector functions. See, for example, U.S. Pat. 4,816,397, European Patent Office publications EP 173,494 and 239,400 and PCT publication WO 89/07142. Such procedures can also be quite laborious and have had only limited experimental validation. Even with these procedures one may still be faced with a recombinant IgG antibody not having sufficient avidity to initiate

biologically important effector functions, or with IgM molecules which have a desired therapeutic activity but suffer from the general disadvantages associated with IgMs as mentioned above.

The avidity of IgG antibodies could be improved by increasing the valency of the molecule to greater than two. More interactions between antibody and antigen would result in tighter binding and would stabilize the antibody-antigen interaction, generally an important attribute for therapeutic use. IgG antibodies of high avidity (via multivalent attachment) and which have the desired effector functions would be greatly preferred over comparable antibodies of low avidity, but to date antibodies having these characteristics have not been described.

Accordingly, what is needed in the art is a means for producing high avidity IgG antibodies having desired effector functions while avoiding many of the difficulties inherent in working with IgMs. Quite remarkably, the present invention fulfills this and other related needs.

Summary of the Invention

Homoconjugated antibodies possess increased therapeutic effectiveness when compared to the corresponding parental antibody monomer. This activity may be due to, inter alia, interactions of higher avidity and increased effector functions. Accordingly, antibodies which bind to the same antigen, and more particularly to the same antigenic determinant, are covalently bonded via cross-linking to one another by synthetic chemical coupling to produce such homoconjugates. Generally, the homoconjugates comprise

at least two to three antibody molecules, typically of the IgG class. The antibodies are preferably monoclonal antibodies, and may be any of a variety of species. For administration to humans the antibodies will usually be human or murine in origin or have human constant regions.

Accordingly, pharmaceutical compositions are provided which comprise a pharmaceutically acceptable carrier and at least two IgG antibody molecules, which bind to substantially the same antigenic determinant, chemically bonded to one another by synthetic covalent linkage. The homoconjugated antibodies and pharmaceutical compositions thereof can be used therapeutically in methods of treatment of antigen related diseases to, e.g., protect against infection, such as by E. coli or group B streptococci, inhibit the growth of tumors, including breast and other tumors, regulate the immune response, and the like. As homoconjugates of IgG antibodies are able to pass the placenta the preparations can be used to treat a fetus in utero.

In another related aspect the invention provides a substantial improvement in methods for therapeutic administration of monoclonal antibodies to a patient for treatment of an antigen related disease. The improvement comprises administering to the patient covalently cross-linked homoconjugated monoclonal antibodies having at least two IgG antibody molecules which bind to the same antigenic determinant of the antigen related to the disease. In preferred embodiments the antibodies are cross-linked via disulfide bonds.

Brief Description of the Figures

Fig. 1 shows chromatograms of FPLC profiles of the IgG homoconjugate mixtures, with retention time along x-axis and A_{280} along y-axis; Peaks labeled A, B and C, represent trimer, dimer and monomer fractions, respectively;

Fig. 2 illustrates the increased binding activity in EIAs of homoconjugates (dimers or trimers) compared to initial monomers of monoclonal antibody D3, a human IgG monoclonal antibody which binds to the group carbohydrate of group B streptococci;

Fig. 3 illustrates the increased binding activity of homoconjugates (dimers or trimers) compared to initial monomers of monoclonal antibody 5E1-G, a human IgG monoclonal antibody which binds to the capsular carbohydrate of E. coli K1;

Fig. 4 illustrates the increased binding activity in EIAs of homoconjugates (dimers) compared to initial monomers of BR64, a murine IgG monoclonal antibody which binds to a human breast tumor associated antigen;

Fig. 5 illustrates the comparative binding activity of homoconjugated chimeric BR96 antibody against tumor cell lines, where Fig. 5A shows binding activity against human breast tumor cell line H3760B, Fig. 5B shows binding activity against human lung tumor cell line H2707, Fig. 5C shows binding activity against human lung tumor cell line H2987, and Fig. 5D is binding activity against human breast tumor cell line H3396;

Fig. 6 shows the increased opsonic activity against group B streptococci by dimer and trimer homoconjugates of human monoclonal antibody D3 compared to the initial IgG monomer;

Fig. 7 shows the enhanced opsonophagocytosis by monoclonal antibody D3 homoconjugates against group B

streptococcal strains M94 and I334 compared to the activity by the D3 monomer antibody;

Fig. 8 shows the increased opsonic activity against E. coli K1 of dimer and trimer homoconjugates of human monoclonal antibody 5E1-G compared to the initial IgG monomer;

Fig. 9 depicts enhanced opsonophagocytosis conferred by homoconjugates of monoclonal antibody 5E1-G against strains H16 and A14 of E. coli K1 compared to the antibody monomer;

Fig. 10 shows increased complement dependent cytotoxicity against breast tumor cell line H3630 by dimer homoconjugates of monoclonal antibody BR64 compared to the initial IgG monomer;

Fig. 11 illustrates the cytotoxicity shown by BR96 homoconjugates and monomeric monoclonal antibody against breast tumor cell line H3396; and

Fig. 12 shows the in vivo protection conferred by homoconjugates of monoclonal antibody D3 and control monomer at different concentrations of antibody.

Description of the Specific Embodiments

The present invention provides homoconjugates of monoclonal antibodies against selected antigens, and methods for preparing such homoconjugates. By chemically linking antibody molecules, homoconjugates are prepared which possess increased valency and two or more Fc regions. By this means a variety of effects may be accomplished, including, inter alia, increases in binding avidity, complement fixation, cellular activation, opsonophagocytosis, etc. Thus the invention provides the ability to convert antibodies of perhaps limited in vivo utility to antibodies having characteristics significantly more conducive to a desired therapeutic

activity. For example, homoconjugation may serve to convert an IgG monomer of low binding avidity to one of higher avidity and better able to promote effector functions that were perhaps not previously attainable.

By homoconjugate is meant the covalent association or linking of two, three or more antibody molecules which bind to the same antigenic determinant, thereby forming antibody homodimers, homotrimers, etc. The homoconjugates may be prepared from two, three or more different monoclonal antibodies (i.e., those produced by different immortalized cell lines) which bind to the same antigenic determinants (epitopes) on the antigen. The monoclonal antibodies which comprise the homoconjugate may be different (produced by distinct cell lines) but preferably they are the same, i.e., obtained from the same cell line, and thus constitute a relatively homogeneous preparation of monoclonal antibodies with virtually identical antigen binding specificity. By binding to the same or substantially the same epitope is meant to refer to monoclonal antibodies which are capable of reciprocal or non-reciprocal competition with the other for binding to the antigen. One skilled in the art will know how to conduct competition immunoassays, such as by radioimmunoassay or enzyme immunoassay, as generally described in, e.g., U.S. Pat. 3,817,837; Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY (1988); and Day, Advanced Immunochemistry, 2d ed., Wiley-Liss Publications, NY (1990), each incorporated herein by reference.

The Fc regions of the monoclonals used for homoconjugation, or other aspects of the immunoglobulin molecule which do not substantially affect antigen binding specificity, may also be altered to produce desired effector functions. For example, it may be desirable to substitute a Fc domain for protein A binding into a molecule not having that capacity, for ease of

purification or the like. Other substitutions may provide for decreased immunogenicity, increased or decreased complement activation, cell receptor binding, control of catabolic rate, placental and gut transfer, ability to participate in antibody-dependent cellular cytotoxicity, and other aspects of immune regulation. A number of antibody functions have been localized to a constant region domain or domains. See, Paul, Fundamental Immunology, Raven Press, New York, NY, 1984, incorporated herein by reference. A wide variety of techniques are available to produce recombinant immunoglobulins, e.g., U.S. Pat. 4,816,397, European Patent Office publications EP 173,494 and 239,400 and PCT publication WO 89/07142, each incorporated herein by reference. Accordingly, the homoconjugated immunoglobulins may be any of the heavy chains and subclasses thereof. The light chains may be either kappa or lambda.

Particularly preferred in the present invention are homoconjugates of antibodies having gamma heavy chains, so as to form homoconjugated multivalent IgG molecules. Within the IgG subclasses of 1, 2, 3 and 4 (human) and 1, 2a, 2b and 3 (murine), human subclasses 1 and 3 and murine subclasses 1, 2a and 2b are generally preferred for applications requiring maximum complement fixation, binding to monocytes, macrophages and polymorphonuclear cells, and the ability to cross the placenta. The effector functions of human IgG_2 and IgG_4 antibodies may also be substantially increased by the homoconjugation procedures described herein.

It is also contemplated that under certain circumstances, depending on the intended use, antibodies having alpha, mu, epsilon or delta type heavy chains may also be employed for homoconjugation as described herein.

The binding affinity of the antibodies for use in homoconjugates will vary, but will generally be at least 10^{-4} M, typically at least about 10^{-6} M to 10^{-7} M,

and preferably at least about 10^{-8} to 10^{-9} M or greater. The avidity of the homoconjugates prepared from such antibodies should generally be at least about 10^{-6} M to 10^{-7} M, and preferably at least about 10^{-8} to 10^{-10} M or greater. Means for determining affinity and avidity are known, as described in Day, Advanced Immunochemistry, supra. While the homoconjugates may have quantitative increases in avidity, generally the homoconjugates should also have qualitative increases in avidity and effector functions, e.g., those evidenced by antigen binding tests and other functional assays as described herein and as will generally be known to one of ordinary skill in the art.

The homoconjugated immunoglobulins may be of any species or combination thereof from which monoclonal antibodies may be prepared. Although it has generally been relatively easy to produce murine monoclonal antibodies of a desired antigen binding specificity, it has been much more difficult to produce human monoclonal antibodies of the desired specificity and having the desired constant region properties. Human monoclonal antibodies are preferable for many applications, especially in vivo diagnosis and therapy of humans to minimize their recognition as foreign by a patient's immune system.

While murine and human immunoglobulins are most commonly produced, monoclonal antibodies or portions thereof originating with other species, such as lagomorpha, bovine, ovine, equine, porcine, avian or the like may be employed. It should be understood that the monoclonal antibody art and genetic engineering techniques have advanced sufficiently such that antibody sequences of one species may be interchanged with those of another species. Thus, as used herein, a "human" antibody, for example, refers to one that is substantially human in origin but may also contain some non-human and/or non-immunoglobulin sequences.

Similarly, when referring to immunoglobulin, used synonymously herein with antibody, it will be understood that some non-immunoglobulin sequences may be present in the molecule while retaining the ability to bind antigen. Immunoglobulin refers to both whole immunoglobulins and binding fragments thereof.

The antibodies which are used for homoconjugation may be substantially monospecific, i.e., relatively pure preparations of substantially homogeneous antibodies obtained from polyclonal antisera, or may be monoclonal antibodies. Monoclonal antibodies which bind to a desired antigen or epitope thereof are obtained from an established cell line which secretes them. The antibody-producing cell line may be isolated from B cells of several species using conventional fusion, viral transformation or other immortalization techniques well known to those skilled in the art. For instance, human monoclonal antibodies may be generated using Epstein-Barr virus (EBV) transformation, hybridoma fusion techniques, or combinations thereof. See, for example, Kozbor et al., Proc. Natl. Acad. Sci. USA 79:6651 (1982), and U.S. Pat. Nos. 4,464,465 and 4,624,921, which are incorporated herein by reference. By monoclonal antibody is meant an antibody produced by a clonal, immortalized cell line separate from cells producing antibodies with a different antigen binding specificity. Thus such monoclonal antibodies are produced and isolated from other monoclonal antibodies and, accordingly, in substantially pure form (relative to other antibodies) and at a concentration generally greater than normally occurring in sera from the animal species which serves as the B cell source.

Thus, it should be understood that the invention is not limited by the antigen binding specificity of the particular homoconjugates exemplified herein, but rather, it can be used in the treatment of a variety of antigen related diseases, particularly those

for which monoclonal antibodies have been therapeutically administered. By antigen related disease is meant a disease whose manifestation coincide with the clinical presence of a foreign antigen (e.g., bacteria, virus, tumor or tumor associated antigen) or self antigen (as with autoimmune diseases). A wide variety of monoclonal antibodies have been described in the technical and patent literature, many of which are publicly available from cell depositories, such as the American Type Culture Collection, 12301 Parklawn Dr., Parkville, MD 20852, whose catalogue, ATCC Catalogue of Cell Lines and Hybridomas, 6th ed. (1988), is incorporated herein by reference. Representative examples of monoclonal antibodies are described in, e.g., U.S. Pat. Nos. 4,596,769, 4,689,299, 4,753,894, 4,834,975, 4,834,976, 4,925,800, and 4,958,009, each of which is incorporated herein by reference. The methods described herein provide the ability to produce novel cross-linked homoconjugates from immunoglobulins obtained from such cell lines.

The chemically linked homoconjugated immunoglobulins will be produced by chemical conjugation of antibodies using well known laboratory procedures, such as by employing cross-linking reagents. By chemically linked is meant that the immunoglobulin molecules are synthetically linked, i.e., not produced as such by a cell, to one another by covalent bonds. A preferred method of conjugation is the formation of at least one covalent bond between the immunoglobulin molecules.

The immunoglobulin molecules are complexed or chemically bonded together by any of a variety of well known chemical linking procedures. The Fc regions or Fab regions may serve as the site of the linkage. The linkage may be direct, which includes linkages containing a synthetic linking group, or indirect, by which is meant a link having an intervening moiety, such as a protein or

peptide, e.g., plasma albumin, or other spacer molecule. For example, the linkage may be by way of heterobifunctional or homobifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, N-succinimidyl 3-(2-pyridydithio) propionate (SPDP) and derivatives, bis-maleimide, 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), cross-linking without exogenous cross-linkers by means of groups reactive with the individual molecules, such as carbohydrate, disulfide, carboxyl or amino groups via oxidation or reduction of the native protein, or treatment with an enzyme or the like. Methods for chemically cross-linking antibody molecules are generally known in the art, and a number of hetero- and homobifunctional agents are described in, e.g., U.S. Pat. Nos. 4,355,023, 4,657,853, 4,676,980, 4,925,921, and 4,970,156, and in Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Press, 1988, Cold Spring Harbor, NY and ImmunoTechnology Catalogue and Handbook, Pierce Chemical Co. (1989), each of which patents and publications is incorporated herein by reference. In general, such synthetic cross-linking should not substantially affect the antigen binding region of the molecules nor the desired effector functions.

Detection and purification of the homoconjugated immunoglobulins may be accomplished by a variety of techniques, including liquid and affinity chromatography, gradient centrifugation, and gel electrophoresis, among others. Increased activity of the homoconjugates may be measured by quantitative antigen binding assays, antibody competition experiments, opsonophagocytic assays, complement dependent cytotoxicity assays, and the like. These techniques are familiar to those skilled in the art, and are described in, for example, Harlow and Lane, supra.

Homoconjugated antibody preparations with increased binding ability will likely be useful in the

treatment and diagnosis of a wide variety of conditions referred to herein as antigen related diseases. The homoconjugates will offer significantly improved therapeutic and diagnostic characteristics compared to the unconjugated monomeric antibody. Due to the increased avidity of the homoconjugates, it is now possible in certain instances to convert a previously non-protective or weakly protective IgG antibody to be protective against infection or tumors, for example, or to act as an immunomodulator by potentiating or otherwise regulating a host's immune response to a particular antigen. Where an IgM antibody to an antigen or particular epitope of the antigen is protective and a monomeric IgG antibody is non-protective or weakly protective, a homoconjugate produced using the methods described herein may provide sufficient avidity to confer significant protection against infection, cell killing, etc. For instance, an IgG dimer or trimer homoconjugate may possess therapeutic anti-infective qualities that may be found with certain multivalent antibodies such as IgMs, but also have qualities inherent to IgG monomers, such as their ability to cross the placenta, to bind to macrophages and PMNs, and the lack of a requirement for complement to mediate opsonization. The IgG homoconjugates may possess other attributes typically associated with IgGs, such as ease of purification, increased stability, increased shelf life, and increased half-life in vivo.

Although the homoconjugate preparations will be useful against a range of targets, such as bacterial and viral antigens, depending of course on the particular specificity of a homoconjugate's antigen binding region, they will be especially useful where the killing of mammalian cells is required. For example, the homoconjugates can be used for the treatment of cancer cells which display particular tumor-associated antigens (e.g., breast or lung tumor associated antigens), the

inhibition or killing of mammalian cells infected with viruses or bacteria or cells which express antigens associated with a particular autoimmune disease. The homoconjugates can also be used to eliminate selected cells from bone marrow or in the immunosuppression of graft recipients, etc.

Of course, it is understood that the present invention is not limited to antibody homoconjugates which are protective or show other such functional attributes in vivo, as increased avidity also makes feasible an array of diagnostic procedures perhaps not otherwise available to a bivalent monomer of low affinity and/or low avidity.

The ability of the resultant antibodies to inhibit a tumor, such as a breast or lung tumor, to act as an immunomodulator, or to protect against challenge by a pathogen, for example, can be measured in a wide variety of in vitro and in vivo systems, as will be known to the skilled artisan. An exemplary protocol for protection against E. coli K1, using a homoconjugated antibody which was non-protective or weakly protective as an IgG, appears in Example III below.

The novel homoconjugates of monoclonal antibodies and pharmaceutical compositions prepared therefrom are particularly useful for administration for prophylactic and/or therapeutic treatment of an antigen-related disease. Preferably, the pharmaceutical compositions can be administered parenterally, i.e., subcutaneously, intramuscularly or intravenously, or orally. Thus, this invention provides compositions for parenteral administration which comprise a solution of the homoconjugated monoclonal antibody preparations or a cocktail of homoconjugated and monomeric antibodies dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These compositions may be sterilized by

conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc. The concentration of antibody in these formulations can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected, the condition being treated, e.g., an infectious disease such as a group B streptococcal or E. coli infection, a tumor, such as breast carcinoma, etc., and the subject being treated, i.e., an adult, child or neonate.

Thus, a typical pharmaceutical composition for intravenous infusion to treat an infection in an adult could be made up to contain 250 ml of sterile Ringer's solution, and about 100 mg to 10 grams of antibody. Actual methods for preparing parenterally or orally administrable compounds will be known or apparent to those skilled in the art and are described in more detail in for example, Remington's Pharmaceutical Science, 16th ed., Mack Publishing Company, Easton, PA (1982), which is incorporated herein by reference.

The compositions containing the present homoconjugated antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease,

i.e., infection, tumor, etc., the age of the patient and the general state of the patient's immune system. Generally, the amounts will range from about 0.1 to about 50 mg of antibody per kilogram of body weight per dose, with dosages of from 5 to 25 mg of antibody per kilogram per patient being more commonly used. It must be kept in mind that the materials of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and the possibility of lower "foreign substance" rejections which may be achieved by, e.g., administering allogeneic homoconjugated antibodies or chimeric homoconjugated antibodies made feasible by this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or cocktails thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend on the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per kilogram, especially 0.5 to 2.5 mg per kilogram. A preferred prophylactic use is for treatment of fetuses and neonates at risk from infection through their mothers. When treatment is dependent on passage through the placenta, the dosage may require adjustment to reflect the percentage of antibody which is able to pass from the blood of the pregnant female to that of the fetus.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a

quantity of homoconjugated antibody sufficient to treat the patient.

The homoconjugated antibodies of the invention may also find several uses in vitro. By way of example, the homoconjugated IgG antibodies of Example I below can be used for detecting the presence of group B streptococci or E. coli K1, for vaccine preparation, or the like.

For in vitro diagnostic purposes, the antibodies may be either labeled or unlabeled. Unlabeled homoconjugated antibodies may find particular use in agglutination assays, or they may be used in combination with other labeled antibodies (second antibodies) that are reactive with the homoconjugated antibodies, such as antibodies specific for the Fc regions. Alternatively, the antibody may be directly labeled. A wide variety of labels may be employed, such as radionuclides, particles (e.g. gold, ferritin, magnetic particles, red blood cells), fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are known to those skilled in the art, such as competitive and sandwich assays as described in, e.g., U.S. Pat. 4,376,110, incorporated by reference herein, and Harlow and Lane, supra.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of the presence of a selected antigen. Thus, the subject antibody compositions of the present invention may be provided, usually in lyophilized form in a container, either alone or in conjunction with additional antibodies. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than

about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about .0001% wt., based on the antibody concentration. Frequently it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% of the total composition. Where a second antibody capable of binding to the homoconjugated antibodies is employed in an assay, this will be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following examples are offered by way of illustration, not by limitation.

EXAMPLE I

Preparation of Monoclonal Antibody Homoconjugates

This example demonstrates means for preparing homoconjugates of several representative monoclonal antibodies to selected tumor and bacterial antigens. The homoconjugates were then tested in functional assays described in the examples which follow.

Homoconjugates of the following monoclonal antibodies were prepared: Monoclonal antibody D3, a human IgG_1 antibody which binds to the group B carbohydrate of group B streptococci. 5E1-G, a human IgG_1 monoclonal antibody which binds to the capsular carbohydrate of *E. coli* K1. BR64, a murine IgG_1 monoclonal antibody which binds to human carcinoma associated antigen, including colon, breast, ovary and lung carcinomas. BR64 is on deposit with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, M.D, as ATCC No. HB 9895. And BR96, also on deposit with the American Type Culture Collection as ATCC No. HB

10036, is an IgG human-murine chimeric IgG monoclonal antibody which binds to human lung and breast tumor associated antigens.

Homoconjugates of each of the antibodies were prepared using maleimidobutyrloxysuccinimide and iminothiolane according to the following protocol. Antibodies (1 mg/ml) were dialyzed overnight against a coupling buffer (0.1M Na_2HPO_4-dibasic, seven-hydrate, 0.1M NaCl, pH 7.5). One milliliter of antibody was thiolated with 2-iminothiolane-HCl (Pierce Chemical Co., 50 µl (0.5 mg) of 2-iminothiolane solution (10 mg/ml in coupling buffer) added while mixing. A second aliquot of the antibody (1 ml) was treated with N-γ-maleimidobutyryloxy-succinimide (GMBS)(Calbiochem, La Jolla, CA), 5 µl (14 µg) of GMBS solution (1 mg in 360 µl dimethylformamide (DMF). Each treated aliquot of antibody was incubated 1 hr. at room temp. and then the antibodies were run over PD-10 columns (Pharmacia) pre-equilibrated in coupling buffer. After a void volume of 2.6 ml total, antibodies were collected in double the original volume. The thiolated and GMBS-treated aliquots of antibodies were then mixed and incubated at room temp. for 5 hrs. The reactions were quenched by adding 1 µl of 25mM β-mercaptoethanol (1 µl in 560 µl coupling buffer) and incubating for 15 min. at room temp. The β-ME was stopped by adding 11 µl (11 µg) N-ethylmaleimide (Sigma Chemical Co., St. Louis) made up to 1 mg/ml in DMF. The homoconjugate preparations were dialyzed overnight in phosphate buffered saline (PBS) and separated by size-exclusion chromatography using Superose-6 and Superose-12 FPLC columns (Pharmacia, Uppsala, Sweden). The chromatograms of the FPLC columns for monoclonal antibodies D3, 5E1-G, and BR64 are shown in Fig. 1.

EXAMPLE II

Binding Activity of Homoconjugates

The ability of the tetravalent and hexavalent monoclonal antibody homoconjugates to bind antigen was compared to the binding activity of the bivalent IgG monomer antibodies. The binding of the anti-GBS homoconjugates was measured against a GBS strain (I334) bound to microtiter wells using poly-L-lysine (PLL). Equivalent protein concentrations of untreated antibody D3 monomer were compared to FPLC fractionated IgG dimer and trimer homoconjugates. Binding was assayed with biotin labeled anti-human gamma-chain specific antibodies. The results are shown in Fig. 2, where the relative binding activities of the dimer or trimer homoconjugate preparations were significantly greater than the initial IgG monomer.

To measure the binding of the anti-E. coli K1 antibody homoconjugates, E. coli strain H16 was bound to microtiter wells using poly-L-lysine. Untreated antibody 5E1-G was compared to homoconjugates of IgG dimer and trimer, prepared as described above. Equivalent protein concentrations of antibodies were reacted with the E. coli. Binding was assayed with biotin labeled anti-human gamma-chain specific antibodies. The results are shown in Fig. 3, where the relative binding activities of the dimer and trimer homoconjugate preparations were significantly greater than the initial IgG monomer.

To measure the binding of the anti-breast tumor antibody, BR64, and homoconjugates thereof, a breast tumor cell line, 3396, was grown adherently to microtiter wells. Untreated antibody BR64 was compared to homoconjugates of IgG dimer and trimer. Equivalent protein concentrations of antibodies were reacted with the breast tumor cells. Binding was assayed with biotin labeled anti-murine gamma-chain specific antibodies. The

results are shown in Fig. 4, where the relative binding activities of the dimer and trimer homoconjugate preparations were significantly greater than the initial IgG monomer.

To measure the binding of the anti-breast and lung tumor antibody, human-mouse chimeric BR96, and homoconjugates thereof, two breast cell lines (H3396 and H3760B) and two lung cell lines (H2987 and H2707) were used as targets. Freshly trypsinized cells were attached to microtiter plates using PLL and the ELISAs performed as follows. PLL, made up at 1 μg/ml in PBS, was adsorbed to Immulon 96-well microtiter plates by incubating 75 μl/well of the PLL solution for 1 hour at room temp. Carcinoma cell lines (cultured in IMDM with 15% FCS) were trypsinized, washed twice, and resuspended in PBS at 2 x 10^5 cells/ml. The PLL treated ELISA plates were washed 3 times with saline/Tween (all wash steps done with a gravity flow wash system). The cell suspension was added at 100 μl/well (about 20,000 cells/well) and incubated for 1 hr at 37°C. The plates were then washed 3 times with saline/Tween. Antibodies were diluted in specimen diluent (5% nonfat dry milk, 100 μl/L Foam A, 0.01% w/v thimerosal in PBS) then added to the ELISA plates (100 μl/well) and incubated for 1 hr at room temp. Following incubation, the plates were washed 3 times with saline/tween, and peroxidase-conjugated goat anti-human or mouse IgG (Tago) diluted in specimen diluent was used as a second step reagent, (100 μl/well) and incubated for 1 hr at room temp. The plates were then washed 5 times with saline/Tween, and tetramethylbenzidine (TMB) chromogen (TMB), diluted 1:100 in buffered substrate, was added (100 μl/well), and plates incubated for 20 minutes. The reactions were stopped with 100 μl/well of 3N H_2SO_4 and the plates read at dual wavelength, 450/630nm.

Untreated monoclonal antibody BR96 was compared to homoconjugated BR96 IgG dimers using approximately equivalent protein concentrations of antibody. The

results, shown in Fig. 5A-D for each of the tested tumor cell lines, indicate that the relative binding activity against the four tumor cell lines by the predominantly dimer homoconjugate preparation was greater than by the initial IgG BR96 monomer.

EXAMPLE III

Increased In Vitro Activity of Homoconjugates

As an indication of *in vivo* effectiveness, the monoclonal antibody homoconjugates to GBS were tested in an *in vitro* opsonophagocytic assay. Homoconjugates to *E. coli* K1 were tested for functional activity in two types of opsonization assays described below. Homoconjugates of BR64 were tested for *in vitro* function in a complement dependent cytotoxicity assay, and homoconjugates of BR96 were tested in a complement independent cytotoxicity assay.

Opsonization of GBS by Homoconjugates of D3

The opsonophagocytic assays for GBS were performed as follows. Bacteria were prepared by inoculating 10 ml of tryptic soy broth (TSB) with 50 µl of an overnight broth culture. The tubes were incubated at 37°C on a shaker for 3 hours at which time 1.5 ml of the culture was centrifuged for 1 min. at 10,000 x g, the spent culture media discarded, and the pellet was suspended in 3.5 ml of Hank's balanced salt solution containing 0.1% gelatin and 5 mM HEPES (HBSS/Gel). The bacterial concentrations were adjusted to about 3×10^4 bacteria/ml by measuring the $O.D._{600}$ and making the appropriate dilutions (approximately 1:50,000). Human neutrophils were isolated according to van Furth and Van Zwet ("*In Vitro* Determination of Phagocytosis and

Intracellular Killing by Polymorphonuclear and Mononuclear Phagocytes," in Handbook of Experimental Immunology, Vol. 2, D.M. Weir, ed., 2nd edition, Blackwell Scientific Publications, Oxford, 36.1-36.24 (1973)) with several modifications. Buffy coat from 5 ml of heparinized blood diluted 1:2 with PBS was underlayed with Lymphocyte Separation Medium and centrifuged. The red blood cell (RBC) pellet was washed once with RPMI 1640 medium and resuspended in an equal volume of 37°C PBS. Twenty-five ml of this suspension was added to 25 ml of 2% dextran (in 37°C PBS) and the contents gently but thoroughly mixed end over end. After a 20 min. incubation at 37°C to allow the RBC's to sediment, the supernatant (containing neutrophils) was removed, washed twice in 4°C PBS, once in HBSS/Gel, and suspended in same to $5x10^7$ neutrophils/ml. For the complement source used with GBS, human serum was thrice adsorbed with live bacteria (Bjornson, A.B. and Michael, J.G., J. Inf. Dis., 130 Suppl:S119-S126 (1974)) corresponding to the organisms used in the assay.

For the assay, into 1.5 ml sterile polypropylene microfuge tubes were added 250 µl antibody (test homoconjugates or monomer) preparation in 10% fetal calf serum in HBSS/gel with HEPES and 100 µl bacterial suspension (about 3 x 10^4 bacteria/ml). After 30 minutes at 37°C, 150 µl containing 75 µl complement, 50 µl neutrophils (5 x 10^7 ml), and 25 µl HBSS/gel were added. The mixtures were incubated on a rotator for 60 minutes at 37°C, after which they were placed into an ice water slurry. After 10 minutes, 20 µl from each tube was added to a 100 mm petri dish containing 3 ml of solidified 0.5% tryptic soy broth agarose, followed by incubation at 37°C. After 18 hours the colonies were enumerated and the data was reported as colony forming units (CFU) for each condition.

The results for homoconjugates of D3 are shown in Fig. 6, where the dimer and trimer required much less

antibody, on a nanogram protein basis, to opsonize the GBS strain tested when compared to the initial IgG monomer.

As a further indication of in vivo effectiveness, homoconjugates prepared with an additional monoclonal antibody to the group B carbohydrate of GBS (D3, produced as generally described in Raff et al., J. Infect. Dis. 163:346 (1991) and PCT patent publication WO 91/06305, each of which is incorporated herein by reference) were tested in in vitro opsonophagocytic assays against two GBS strains, M94 and I334. The results of the assays are shown in Fig. 7, where it is evident that the anti-GBS D3 homoconjugates resulted in increased opsonization of the GBS human clinical isolates. Again, these results suggest that the homoconjugates will significantly increase the in vivo protective activities of the antibodies when compared to the parental IgG monomeric monoclonal antibodies.

Opsonization of E. coli K1 by 5E1-G Homoconjugates

To isolate human neutrophils, heparinized human blood (5 ml) was layered onto 3.0 ml of Mono-Poly Resolving Medium (MPRM, Flow Labs) in polystyrene tubes and centrifuged for 30 minutes at 300 x g at room temp. After centrifugation, three cell layers were evident, with the middle layer containing neutrophils. The serum and top cell layer were removed and discarded, the neutrophils collected and added to a 50 ml tube containing pre-warmed PBS. The neutrophils were centrifuged for 10 minutes at 300 x g at room temp., the supernatant discarded and the cell pellet resuspended with 10 ml tissue culture media (RPMI-1640) containing 0.5% gelatin, and the cell concentration adjusted to 5 x 10^6 cells/ml.

The assays were performed as follows. To luminometer tubes (LKB Nuclear) were added 100 µl

containing appropriate test (5E1-G) or control IgG monoclonal antibody monomer to P. aeruginosa flagella, 100 µl log phase growth bacterial suspension (OD_{660} =0.02), and 100 µl diluted bacteria-adsorbed human serum complement, final concentration 3.3%. The complement was thawed just prior to use and received 5 µl of 2 M $CaCl_2$/ml. The tubes were placed into a prewarmed LKB Luminometer which allows 24 tubes to be run on a continuous reading cycle. After 30 minutes in which the tubes were warmed and periodically mixed, 100 µl of neutrophils (5 x 10^6/ml) and 600 µl of 10^{-4} M Luminol in Hank's Balanced Salt Solution were added. Counting sessions for 25 continuous cycles, which corresponded to ~80 minutes for 24 sample tubes, were initiated. The chemiluminescence intensity was displayed as millivolts (mV) with

$$\text{signal:noise} = \frac{\text{mV values for tubes containing the test antibody}}{\text{average of tubes containing negative antibody.}}$$

The results of the assays are shown in Fig. 8, where it is evident that the homoconjugates resulted in increased opsonization of the E. coli organisms than the initial IgG monomers. The homodimer and homotrimer of 5E1-G were significantly more opsonic than the 5E1-G IgG monomeric form. As the opsonophagocytic assays are typically predictive of in vivo ability to protect animals (see, e.g., U.S. Pat. No. 4,970,070, incorporated herein by reference), these results suggest that the dimer and trimer homoconjugates will significantly increase the in vivo protective activities of the antibodies when compared to the parental IgG monomeric antibody.

As a further confirmation of in vitro efficacy, and thus in vivo activity, the monoclonal antibody homoconjugates to E. coli K1 were tested in in vitro opsonophagocytic assays, as described above, against two

additional E. coli K1 strains, H16 and A14. As shown in Fig. 9, the anti-E. coli K1 homoconjugates resulted in increased opsonization of the human clinical isolates, suggesting that the predominantly dimer homoconjugate preparations will significantly increase the in vivo protective activities of anti-E. coli K1 monoclonal antibodies.

Complement Dependent Cytotoxicity BR64 Homoconjugates

In vitro functional assays were also used to demonstrate the increased functional activity of the anti-tumor antigen homoconjugated monoclonal antibodies. For testing BR64 homoconjugates, target tumor cells (H3630) were labeled with ^{51}Cr by incubation 1 x 10^6 cells/0.3 ml tissue culture media in 100 μCi of ^{51}Cr for 1 hour at 37°C, 6% CO_2. After washing to remove excess ^{51}Cr, 2 x 10^4 labeled cells in 67 μl media (RPMI-1640 plus 15% fetal bovine serum) were added per microtiter plate well. Next, 67 μl of the appropriately diluted test monomer (BR64), a negative control monomer (Mab 96.5), or homoconjugated (dimer) monoclonal antibody was added to duplicate wells. Finally, 67 μl of freshly thawed human serum complement was added to each well, the plates covered with parafilm and incubated at 37°C for 4 hours. After incubation, plates were centrifuged at 400 g for 10 minutes, and 100 μl of supernatant was removed from each well and placed in 12 x 75 mm polystyrene tubes. The tubes were counted in a gamma counter. The following controls were included in each assay:

Table I

Well	Media	Serum	Diluted Antibody	Target Cell
Spontaneous Release	134§	-	-	67
Complement Toxicity	67	67	-	67
Total Incorporation*	134	-	-	67
Maximum Release	67	-	-	67
Antibody Alone	67	-	67	67

§Amounts are expressed as µl/well.

*Prior to incubation of the assay, these are the only wells which contain less than 201 µl, because the wells later receive 67 µl Triton X-100 to lyse labelled target cells.

The percentage kill (% kill) was calculated from the following formula:

$$\frac{\text{[Test (mean CPM)- Hc' control (mean CPM)]}}{\text{Total incorporation [mean CPM - Hc' control (mean CPM)]}} \times 100 = \% \text{ kill}$$

where CPM is counts per minute as average of duplicate samples obtained from measurement in gamma counter and Hc' is Complement Toxicity control.

The results of the assays are shown in Fig. 10, where it is evident that the homoconjugated BR64 resulted in eight times greater killing of the targeted tumor cells than the initial IgG BR64 monomer. The CDC assay is generally predictive of *in vivo* ability to protect animals against tumors. These results suggest that the homoconjugates will significantly increase the utility of such antibodies *in vivo* against tumors, particularly when compared to parental IgG monomer antibodies.

Increased Complement Independent Cytotoxicity of Chimeric BR96 Homoconjugates

Target tumor cells (H3396) at 5 x 10^5 cells/tube were mixed with 100 µl of test antibody and were incubated at 37°C for 30 minutes. Cells were pelleted and mixed with the appropriate concentration of propidium iodide (Sigma, 10 µg/tube). Propidium iodide is a DNA reactive stain that only penetrates the membrane of dead or dying cells. Therefore, by quantitating the number of fluorescent cells within the population, the number of dead cells can be determined (Hellstrom et al., Cancer Res., 50:2183-2190 (1990)). After incubation for 10 minutes, the cells were washed in tissue culture media containing 15% fetal calf serum, resuspended in same, and placed on ice. The cells were analyzed for fluorescence on an EPICS Fluorescence Activated Cell Sorter which quantitates live and dead cells on the basis of fluorescence and size (small and large represent dead and live cells, respectively). The results (Fig. 11) showed that the BR96 homoconjugate dimers were dramatically more effective in killing the tumor cells than the initial monomer. These results suggest that the homoconjugates will significantly increase the utility of such antibodies in vivo against tumors.

EXAMPLE IV

In Vivo Protection Against E. coli K1 Infection in Neonatal Rats Using IgG Homoconjugates

Outbred Sprague-Dawley rat pups less than 48 hours old (housed with their mothers) were injected intraperitoneally with approximately 72 E. coli K1 organisms, and 2 hours later received 1 or 5 μg of dimer homoconjugates of 5E1-G, or 100 μg of monomeric 5E1-G antibody, or control IgG and IgM antibodies. In all experiments, the rat pups were examined daily for symptoms and were scored for survival. The results of the experiments, shown in Table II below, demonstrate that 5 μg of the dimer homoconjugates of 5E1-G antibody protected significantly more animals from death when compared to animals receiving twenty times the amount (100 μg) of monomeric antibody.

Table II

Protection by Homoconjugates Against E. Coli K1 Infections

Antibody (per rat)	Dose (per rat)	n (rats/group)	%Survival (Survivors/Challenged)	p value
5E1-IgM	20 ng	26	100%	<0.001*
5E1-IgG Monomer	100 μg	15	40%	<0.01
5E1-IgG Conjugate	5 μg	14	78%	<0.01
5E1-IgG Conjugate	1 μg	14	29%	<0.05
21B8 (Negative control)	100 μg	24	0	
No antibody control	-	25	0	

*Based on survival in experimental group versus survival in negative control and controls receiving no antibody.

EXAMPLE V

Transplacental Passage of Homoconjugated Antibody to Fetuses of Pregnant Rats

The ability of the homoconjugated IgG antibody to pass through the placenta and into the fetus, and thus into the subsequently delivered offspring, was compared with the monomeric antibody. An infant rat model was used as an animal model. Similar rat models have been used to predict the transplacental passage of antibody and other molecules to human fetuses. See generally, Brambell, Frontiers Biol. 18:234-276 (1970).

Two to three days prior to their anticipated delivery date, pregnant rats were injected intravenously with 40 μg of either monomeric 5E1-G IgG (monomer) (Dams 1 and 2) or homoconjugated dimeric IgG (Dams 3 and 4). Blood samples were collected from the dams two hours after antibody administration and on the day of delivery, and from the neonatal rats just after birth. Total human IgG and human IgG anti-E. coli K1 antibody were determined in each blood sample using individually designed quantitative binding assays (ELISA's). By using anti-human IgG-specific enzyme-labeled secondary antibodies, rat IgG was neither detected nor interfered with the quantitation of the injected human IgG.

The amount of transplacentally passaged antibody was determined as follows. Anti-human gamma chain antibody was attached to microtiter plates using carbonate buffer. After adding diluted serum samples from the dams or pups, binding was assayed with biotin labeled anti-human gamma chain-specific antibodies. Since one group of dams received only conjugated antibody, any human IgG detected in pup sera should be transplacentally passed homoconjugate.

The experiments showed that the monomeric and homoconjugated IgG antibodies were transplacentally

passed with approximately equal efficiency. Therefore, the homoconjugated IgG monoclonal antibody should be useful when administered prophylactically to pregnant females at risk of having a neonate with an increased likelihood of developing a life-threatening infection, such as by E. coli K1 in the case of the present embodiment. The data also support the use of these homoconjugates in transplacental treatment of a variety of other infections and tumors.

EXAMPLE VI

Transplacental Passage of Homoconjugated Monoclonal Antibody to Group B Streptococci

This Example demonstrates the transplacental passage of homoconjugated monoclonal antibody D3.

The experiments were performed as generally described in Example V for the homoconjugated monoclonal antibody to E. coli K1.

The results, shown in Table III, below, indicated that both the monomeric and homoconjugated IgG antibodies were transplacentally passed.

TABLE III: Transplacental Passage of Homoconjugated Antibody From Pregnant Rats to Their Neonates

Source	Time Post Injection	Antibody Injected Homoconjugate	Monomer
Dams	2 Hours	1.45±0.4[a]	2.4±0.3
Dams	3 Days (Day of Delivery)	0.14±0.04	0.11±0.03
Pups	Day of Delivery	0.41±0.1	0.70±0.2

[a] Concentration of human IgG in rat serum (μg/ml)

Accordingly, the homoconjugated IgG monoclonal antibody is useful administered prophylactically or therapeutically to pregnant females likely to deliver a neonate susceptible to developing or already having an infection, such as by group B streptococci or E. coli K1. The present invention also makes possible the use of the homoconjugates in transplacental treatment of a variety of other infections and tumors.

EXAMPLE VII

In Vivo Protection Against Group B Streptococcal Infection With IgG Homoconjugates

This Example describes the use of homoconjugates of the D3 monoclonal antibody to protect against group B streptococcal infection in vivo, consistent with and confirming the results of the in vitro opsonophagocytic assays.

As generally described for the E. coli K1 protection studies described in Example IV above, outbred Sprague-Dawley rat pups less than 48 hours old (housed with their mothers) were injected intraperitoneally with approximately 100 GBS organisms two hours after receiving an intraperitoneal injection of either 20, 4, 0.8 or 0.2 μg of predominantly dimer homoconjugate preparations, 80, 20, or 4 μg of monomeric D3, or control IgG. In the experiments, rat pups were examined daily for seven days and were scored for symptoms and survival. The results from two experiments (data pooled, 25 animals/group), shown in Fig. 12, demonstrate the increased in vivo protective activity against GBS of the dimer homoconjugates of human monoclonal antibody D3 compared

to the initial IgG monomer. As little as 4 μg of homoconjugated dimer protected animals nearly as well as that conferred by 80 μg of monomer.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition which comprises covalently cross-linked homoconjugated monoclonal antibodies having at least two IgG antibody molecules which bind to the same antigenic determinant, and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, the homoconjugated monoclonal antibodies having two antibody molecules.

3. The pharmaceutical composition of claim 1, the homoconjugated monoclonal antibodies having three antibody molecules.

4. The pharmaceutical composition of claim 1, wherein the antibodies are cross-linked by disulfide bonds.

5. The pharmaceutical composition of claim 1, wherein the antibodies are human.

6. The pharmaceutical composition of claim 1, wherein the antibody molecules are murine.

7. The pharmaceutical composition of claim 1, wherein the antibody molecules are murine-human chimerics.

8. The pharmaceutical composition of claim 5, wherein the human antibody heavy chain is an IgG_1.

9. The pharmaceutical composition of claim 1, which is protective against infection due to E. coli K1.

10. The pharmaceutical composition of claim 1, which is protective against infection due to group B streptococci.

11. The pharmaceutical composition of claim 1, wherein the homoconjugated monoclonal antibodies bind to a tumor associated antigen and inhibit growth of breast tumor cells.

12. The pharmaceutical composition of claim 1, wherein the homoconjugated monoclonal antibodies are capable of crossing the placenta.

13. The pharmaceutical composition of claim 1, wherein the constant regions of the light and heavy chains of the antibody molecules are human.

14. The pharmaceutical composition of claim 1, wherein the cross-linked antibodies are derived from the same cell line.

15. A method of treating a patient having a disease related to an antigen, the method comprising administering to the patient a therapeutically effective amount of homoconjugated monoclonal antibodies including at least two covalently cross-linked IgG antibody molecules which bind to the same determinant of the antigen.

16. The method of claim 15, wherein the antigen related disease is group B streptococcal infection.

17. The method of claim 15, wherein the antigen related disease is E. coli K1 infection.

18. The method according to claim 15, wherein the homoconjugated monoclonal antibodies are administered to a pregnant patient and the homoconjugates are able to pass through the placenta into fetal circulation.

19. The method according to claim 18, wherein the homoconjugated monoclonal antibodies are able to treat the fetus for the antigen related disease.

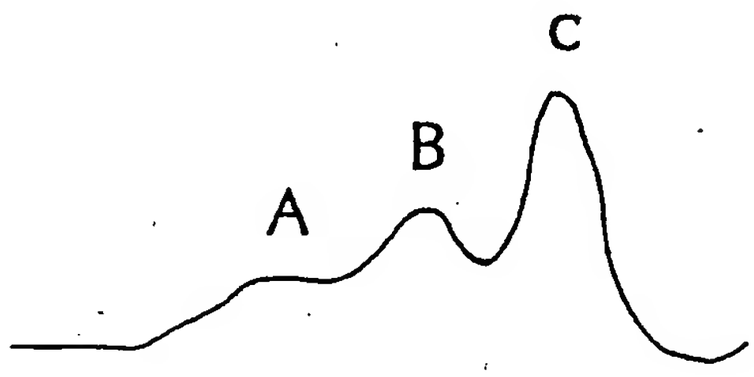
20. The method of claim 19, wherein the antigen related disease is infection by group B streptococci or E. coli K1.

21. The method of claim 15, wherein the antigen related disease is breast tumor and the homoconjugated monoclonal antibodies bind to a breast tumor associated antigen.

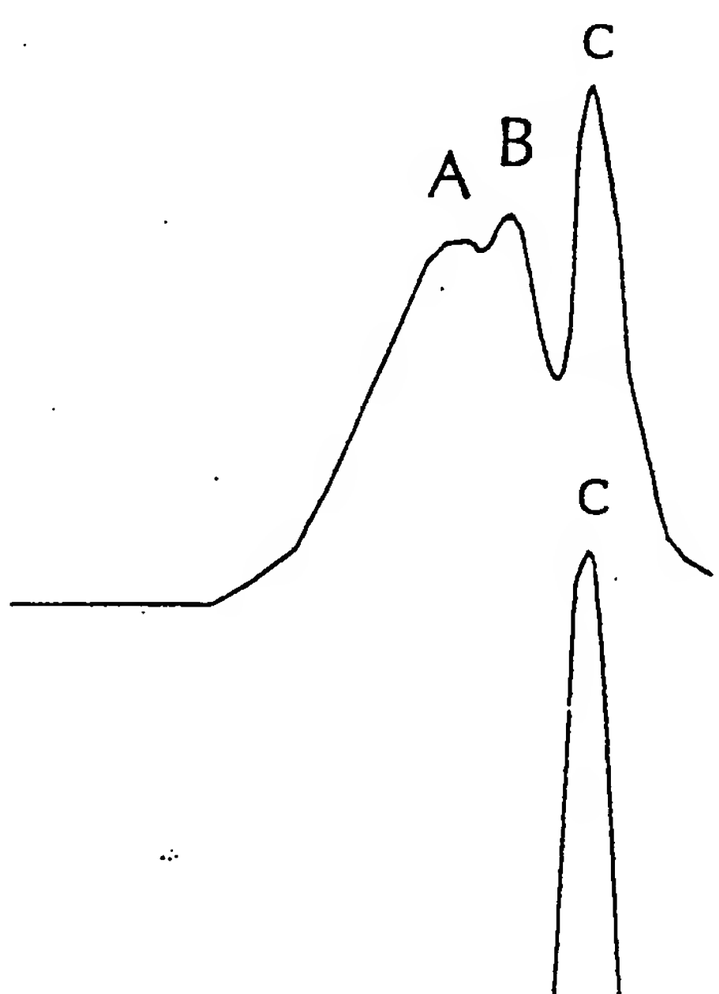
22. In a method for therapeutic administration of monoclonal antibodies to a patient for treatment of a disease related to an antigen, the improvement which comprises administering to the patient homoconjugated monoclonal antibodies including at least two covalently cross-linked IgG antibody molecules which bind to the same antigenic determinant of the antigen.

Chromatograms of Homoconjugate Mixtures

D3 (Anti-GBS)



5E1-G
(Anti-E. coli K1)



BR64
(Breast Carcinoma)

Figure 1

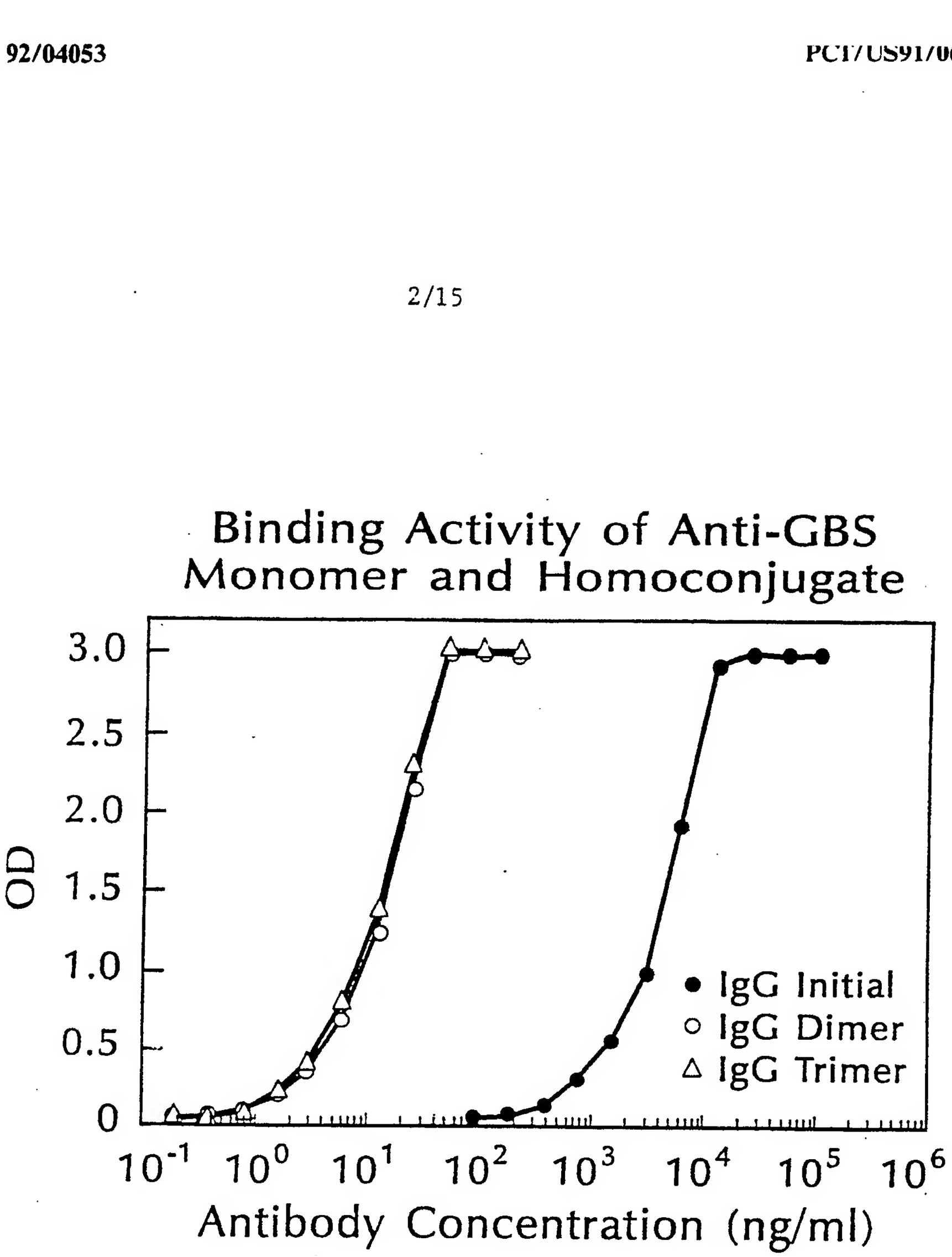

Binding Activity of Anti-GBS Monomer and Homoconjugate
OD
3.0
2.5
2.0
1.5
1.0
0.5
0
IgG Initial
IgG Dimer
IgG Trimer
10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6}
Antibody Concentration (ng/ml)

Figure 2

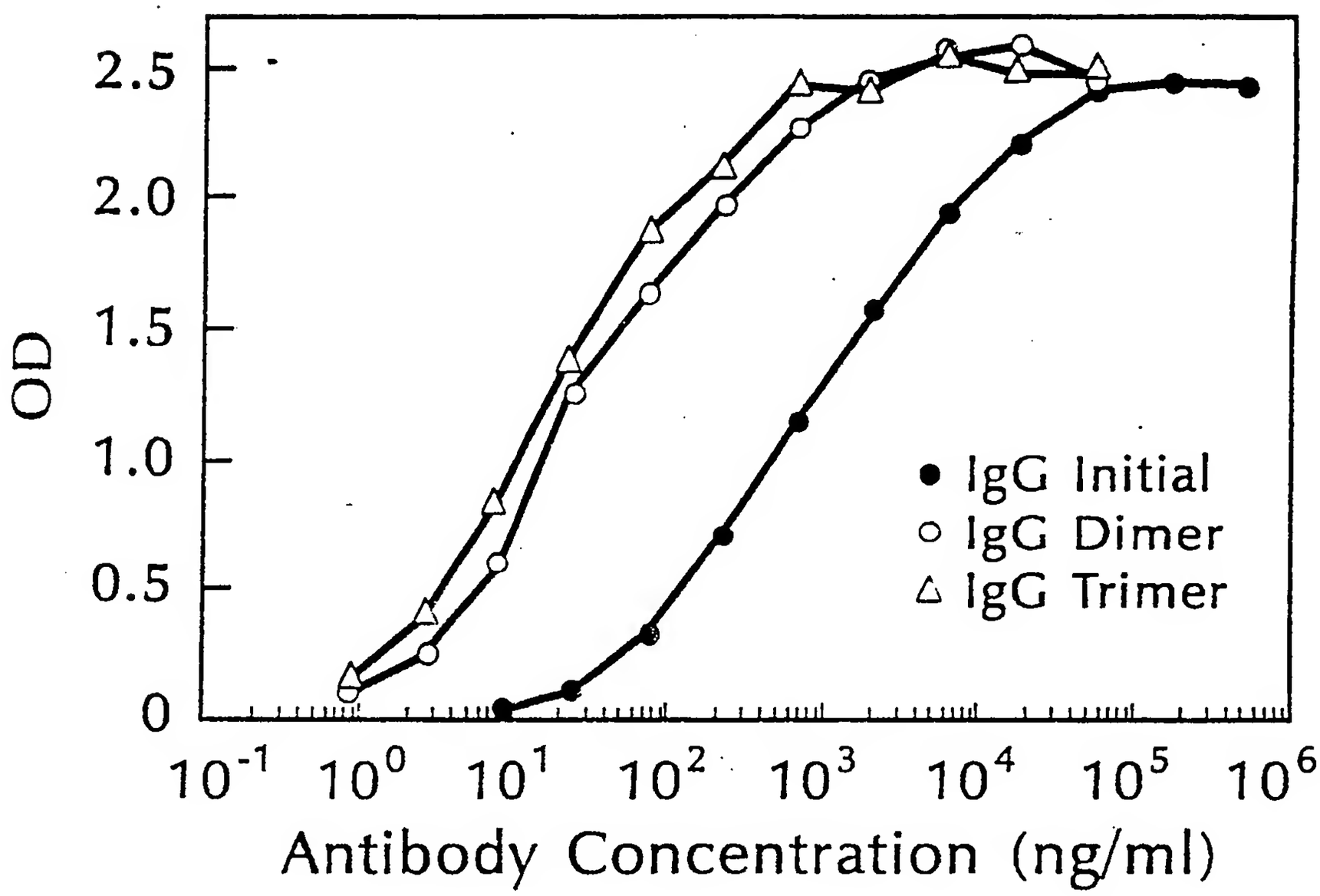

Binding Activity of Anti-E. coli K1
Monomer and Homoconjugate
OD
2.5
2.0
1.5
1.0
0.5
0
IgG Initial
IgG Dimer
IgG Trimer
10-1 10^0 10^1 10^2 10^3 10^4 10^5 10^6
Antibody Concentration (ng/ml)

Figure 3

Binding Activity of Murine Anti-Tumor (BR64) Monomer and Homoconjugate

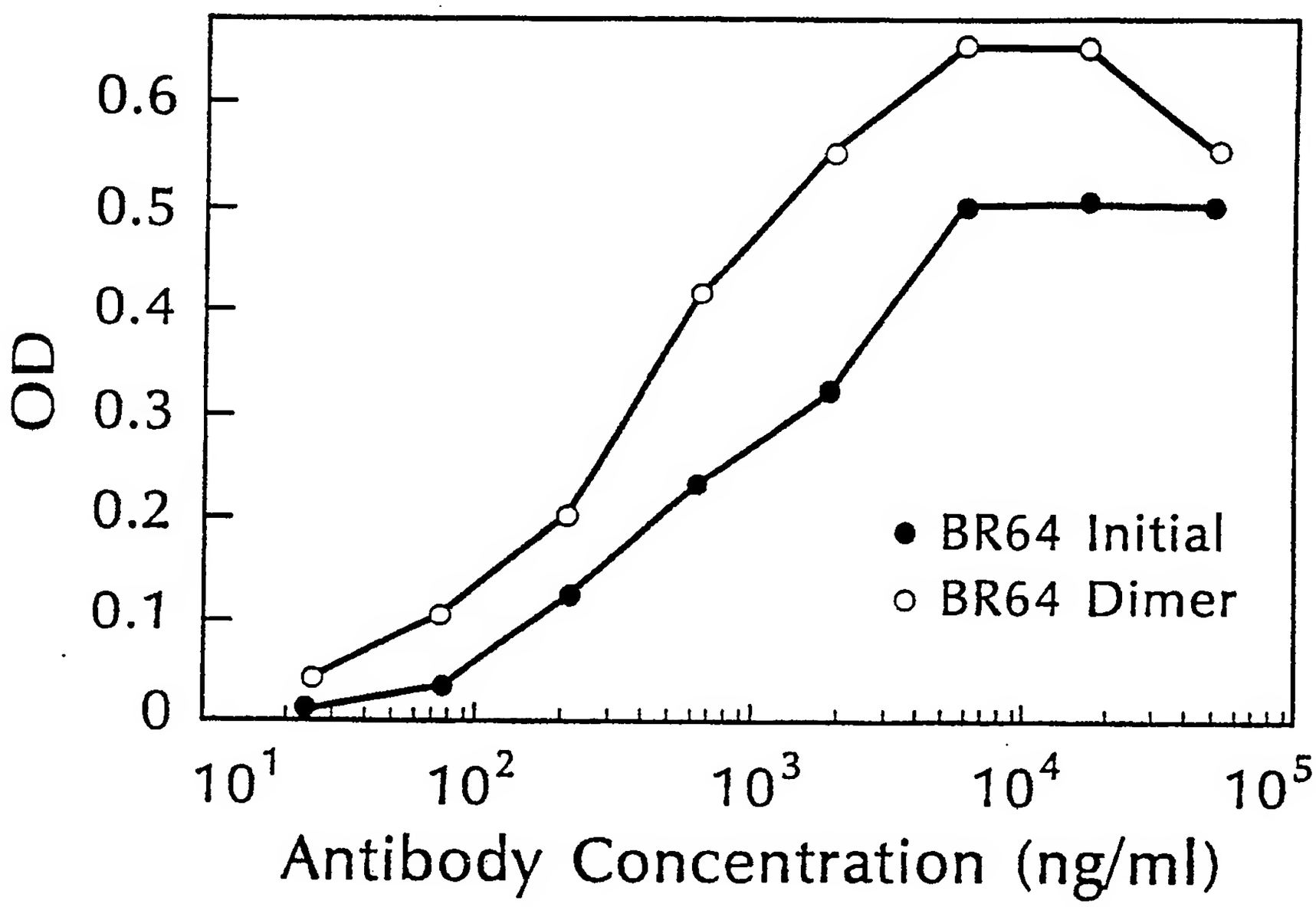


Figure 4

ELISA Binding Activity of BR96 Homoconjugate on PLL-Fixed H3760B Cells

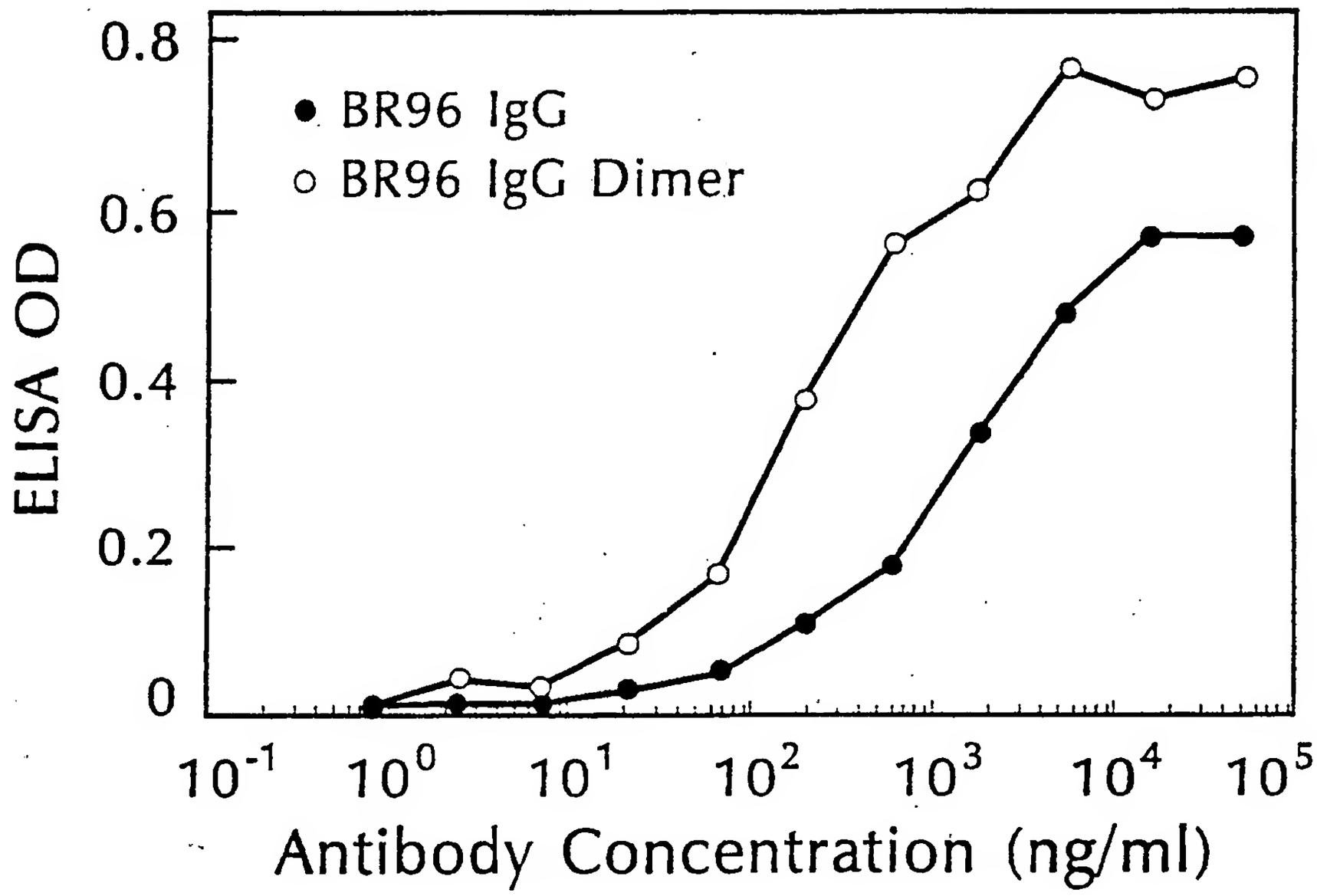


Figure 5A

ELISA Binding Activity of BR96 Homoconjugate on PLL-Fixed H2707 Cells

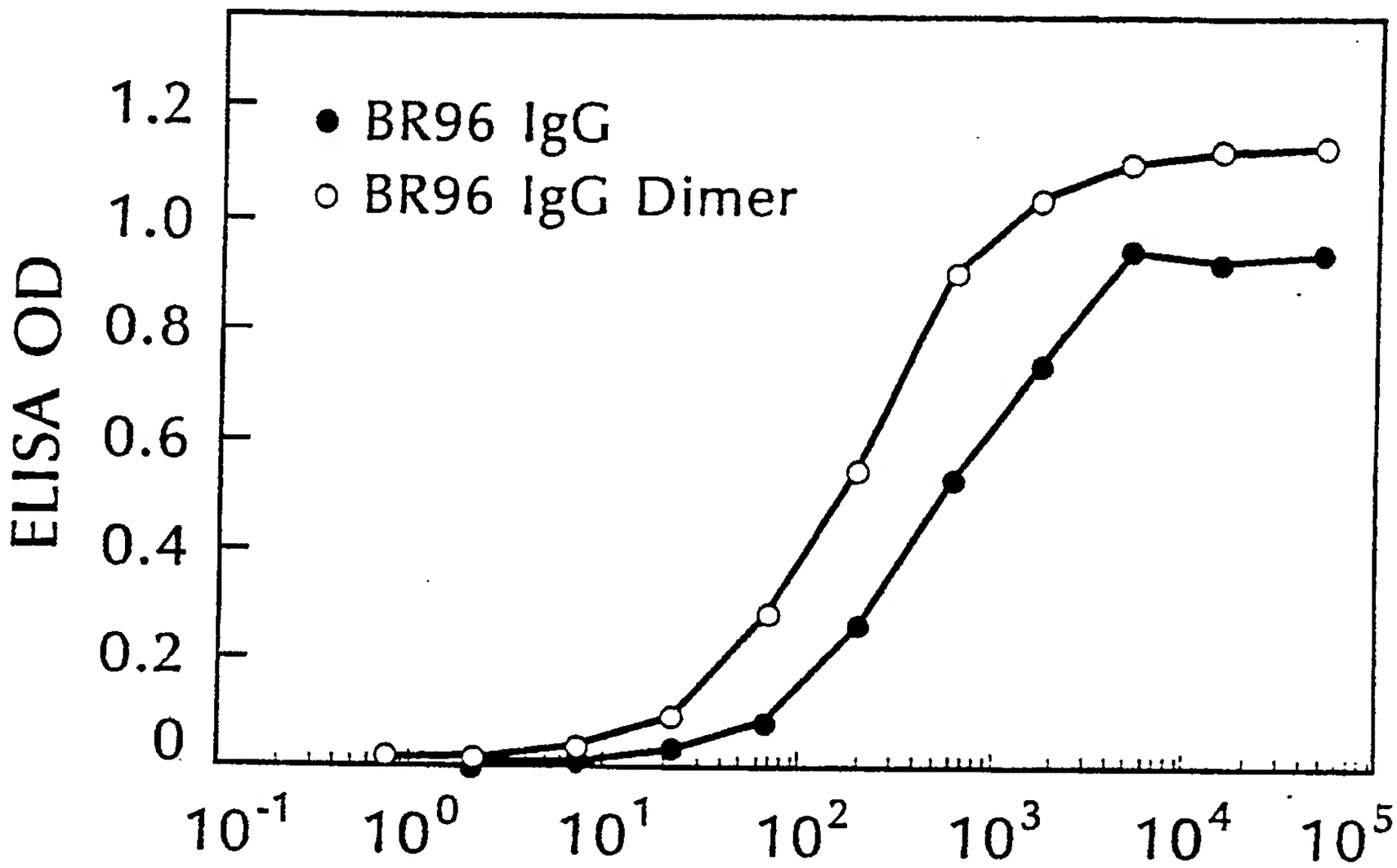


Figure 5B

ELISA Binding Activity of BR96 Homoconjugate on PLL-Fixed H2987 Cells

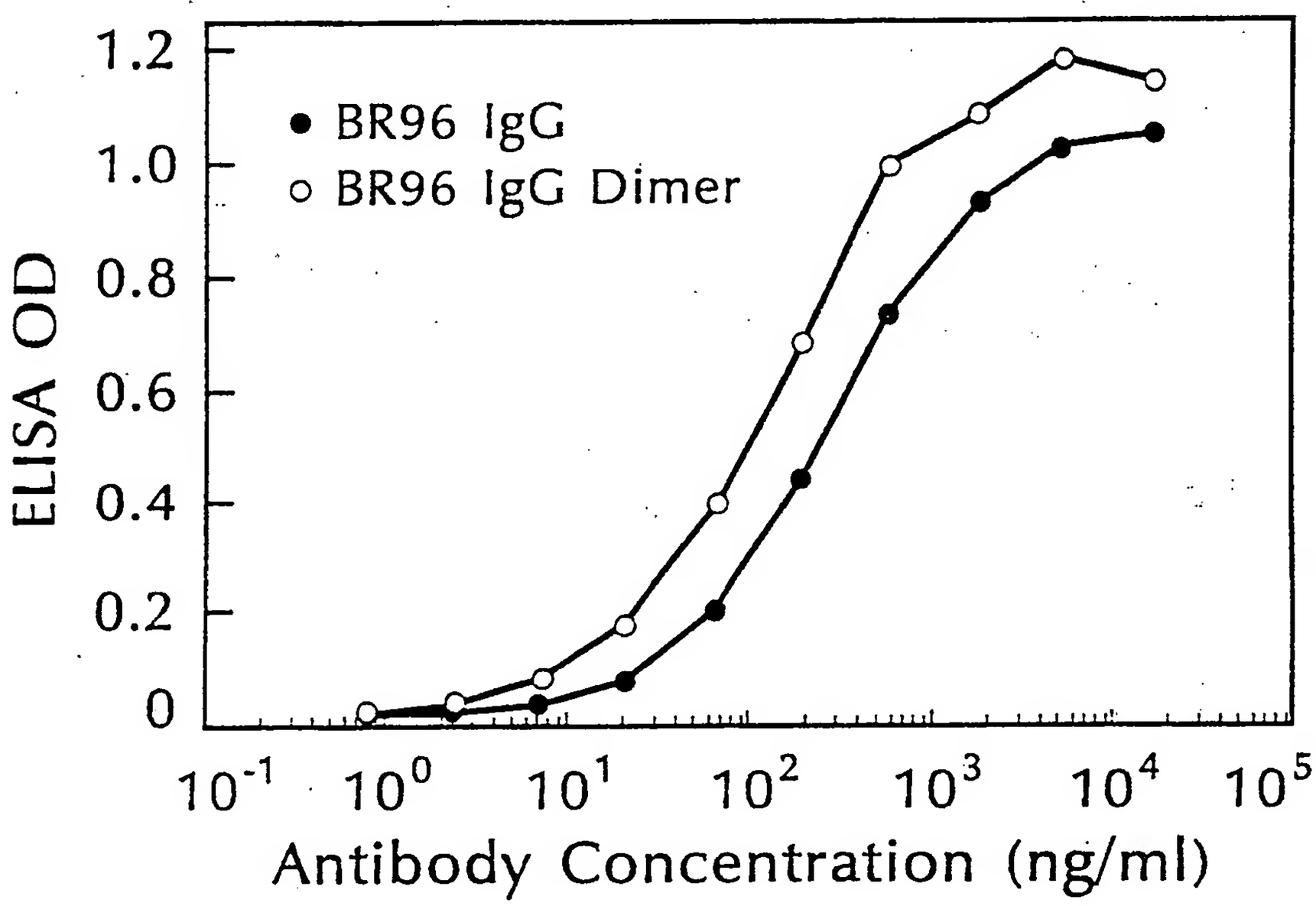


Figure 5C

ELISA Binding Activity of BR96 Homoconjugate on PLL-Fixed H3396 Cells

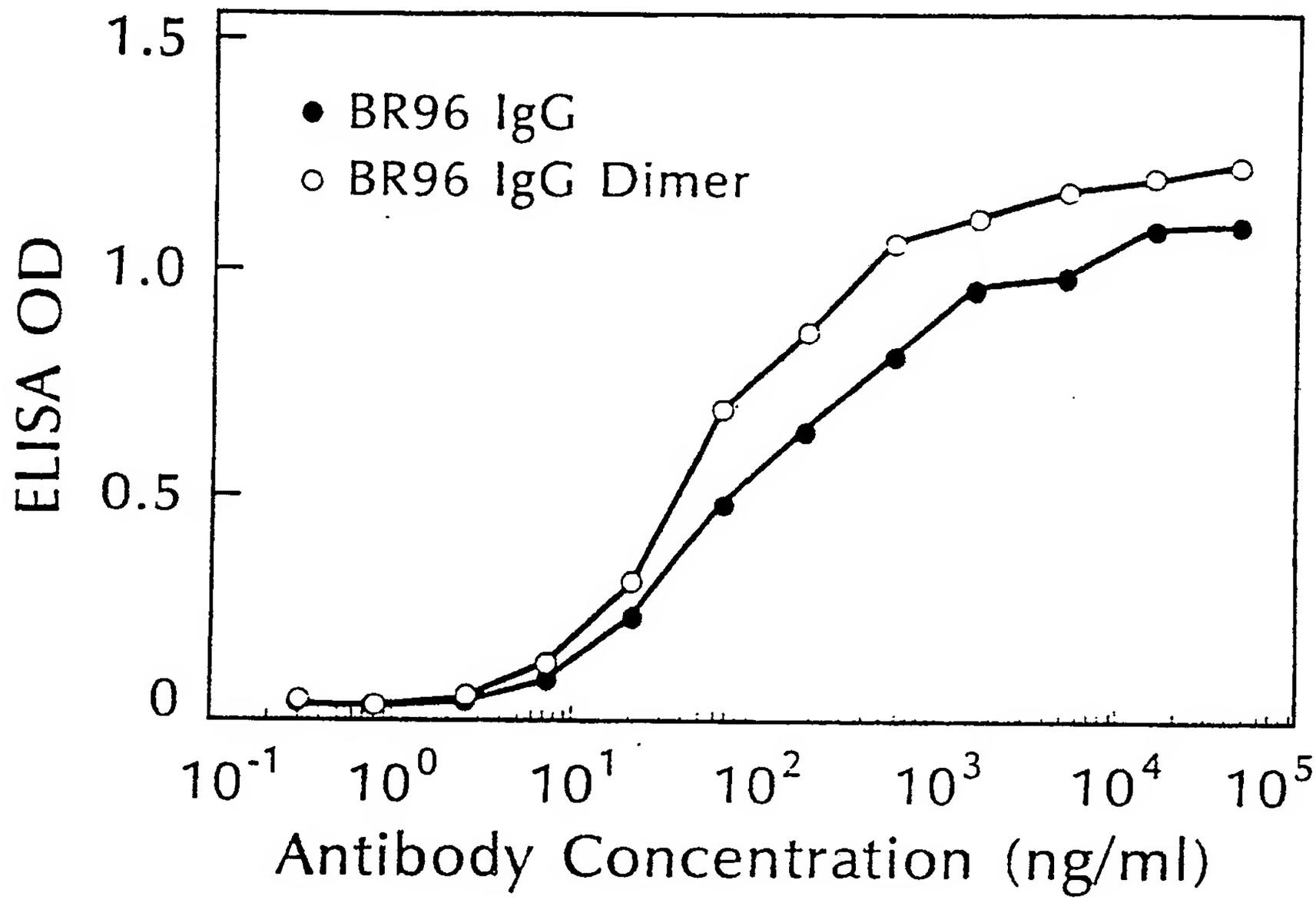


Figure 5D

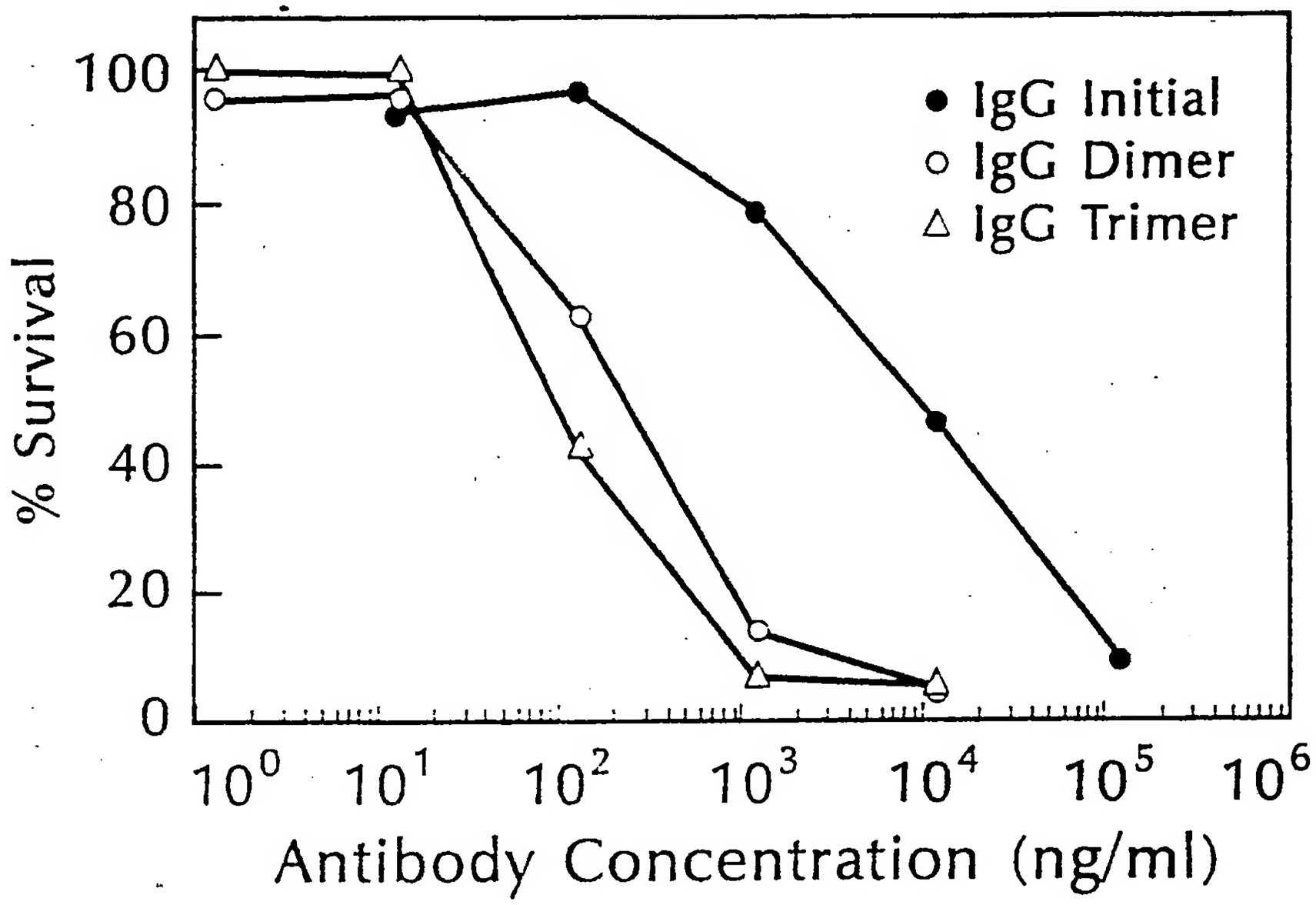

Opsonic Activity of Anti-GBS
Monomer and Homoconjugate
100
80
60
40
20
0
% Survival
IgG Initial
IgG Dimer
IgG Trimer
10^0 10^1 10^2 10^3 10^4 10^5 10^6
Antibody Concentration (ng/ml)

Figure 6

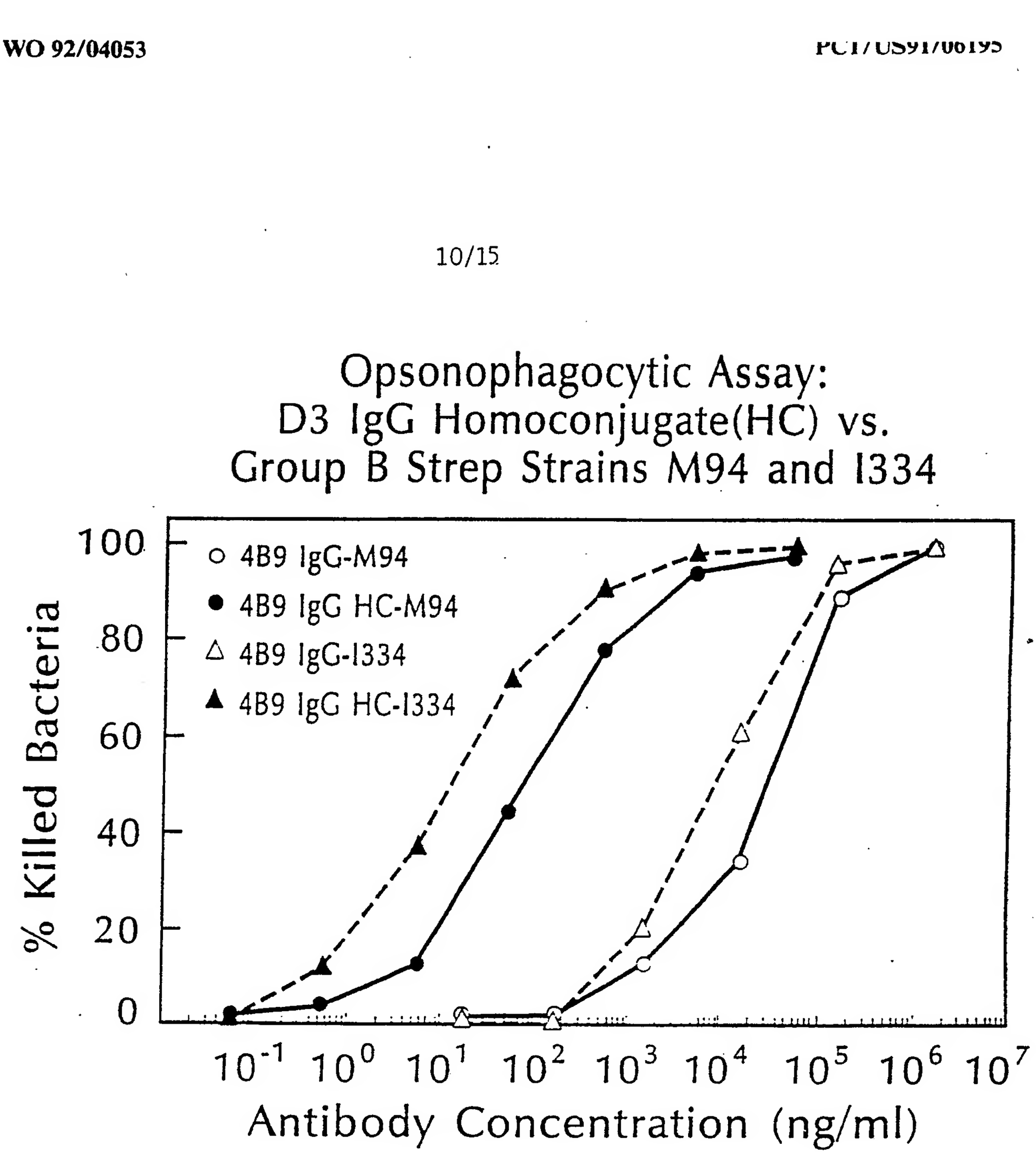

Opsonophagocytic Assay:
D3 IgG Homoconjugate(HC) vs.
Group B Strep Strains M94 and I334
100
80
60
40
20
0
% Killed Bacteria
4B9 IgG-M94
4B9 IgG HC-M94
4B9 IgG-I334
4B9 IgG HC-I334
10-1 10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7
Antibody Concentration (ng/ml)

Figure 7

Opsonic Activity of Anti-E. coli K1 Monomer and Homoconjugate

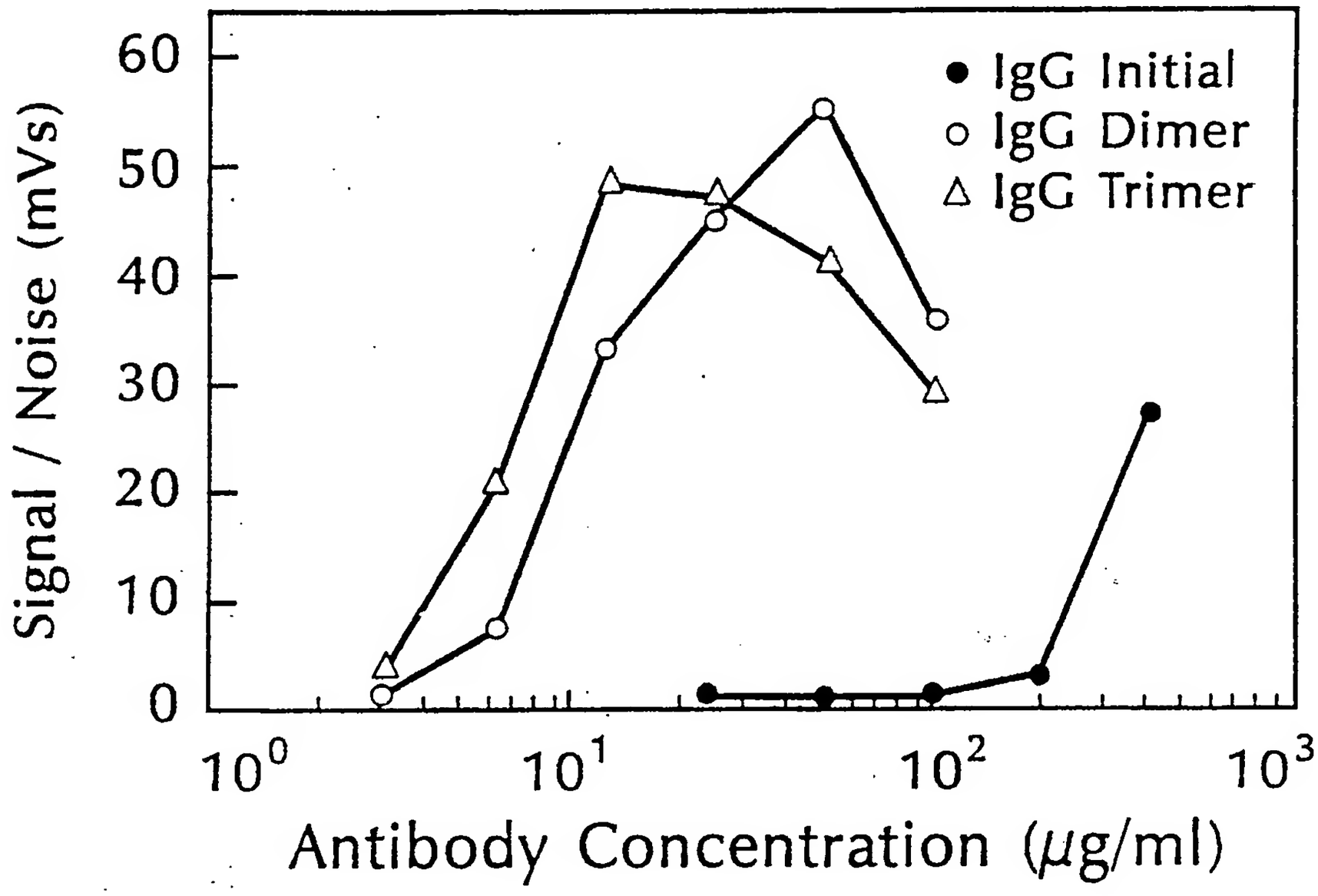


Figure 8

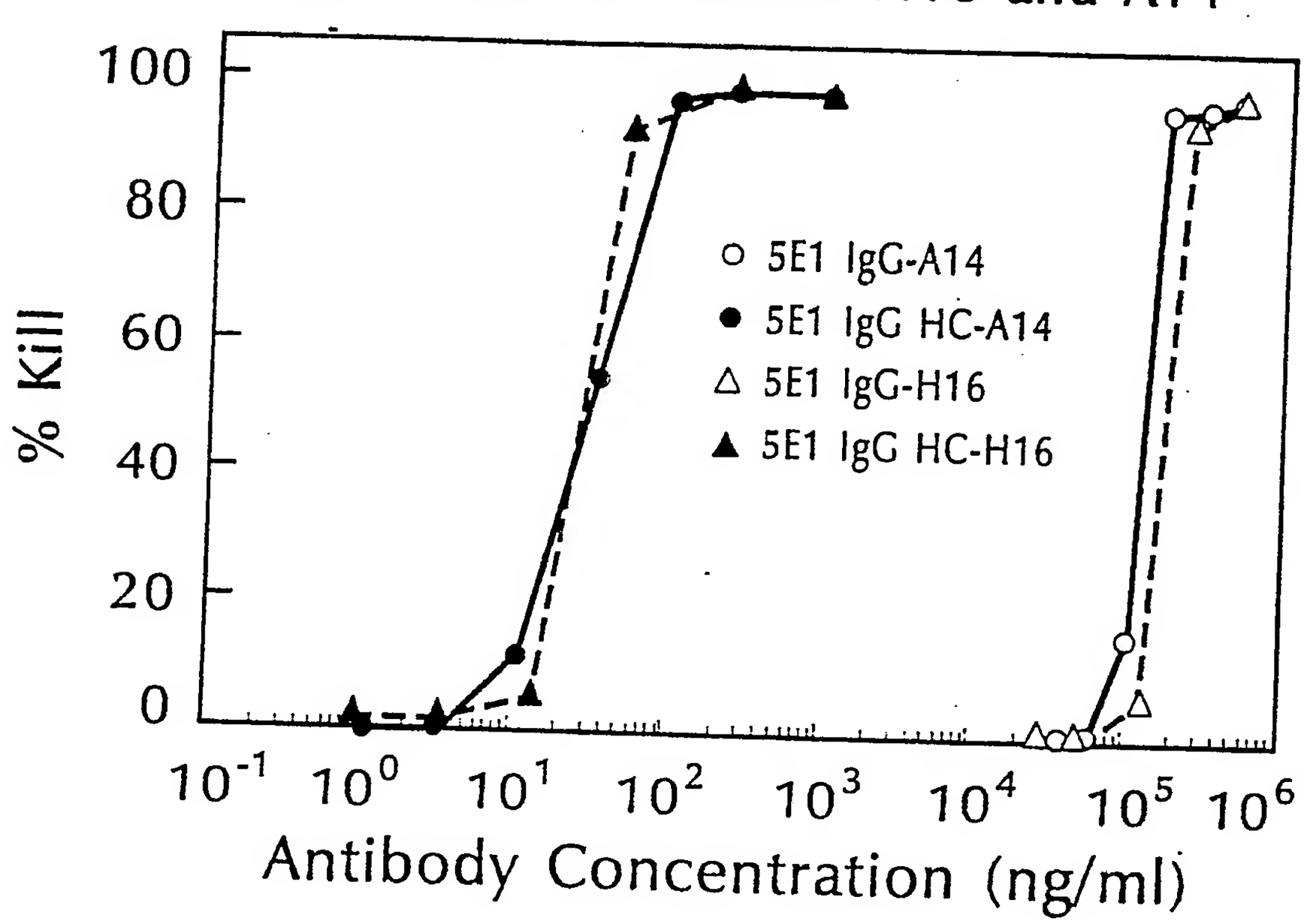

Opsonophagocytic Assay: 5E1 IgG Homoconjugate
vs. E. coli K1 Strains H16 and A14
100
80
60
40
20
0
% Kill
5E1 IgG-A14
5E1 IgG HC-A14
5E1 IgG-H16
5E1 IgG HC-H16
10-1 10^0 10^1 10^2 10^3 10^4 10^5 10^6
Antibody Concentration (ng/ml)

Figure 9

CDC Assay with BR64 Homoconjugate
Using Breast Carcinoma Cell Line H3630

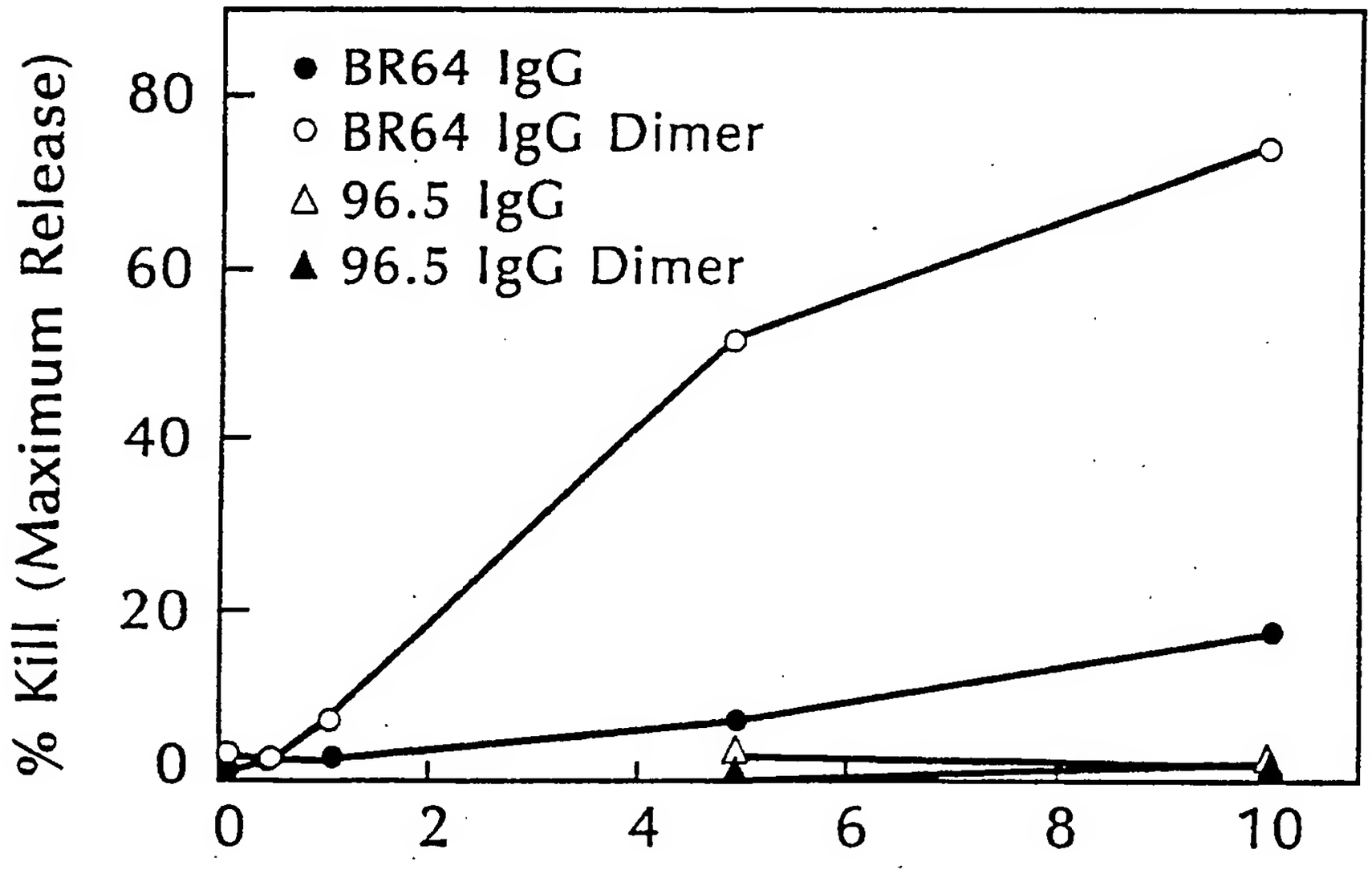


Figure 10

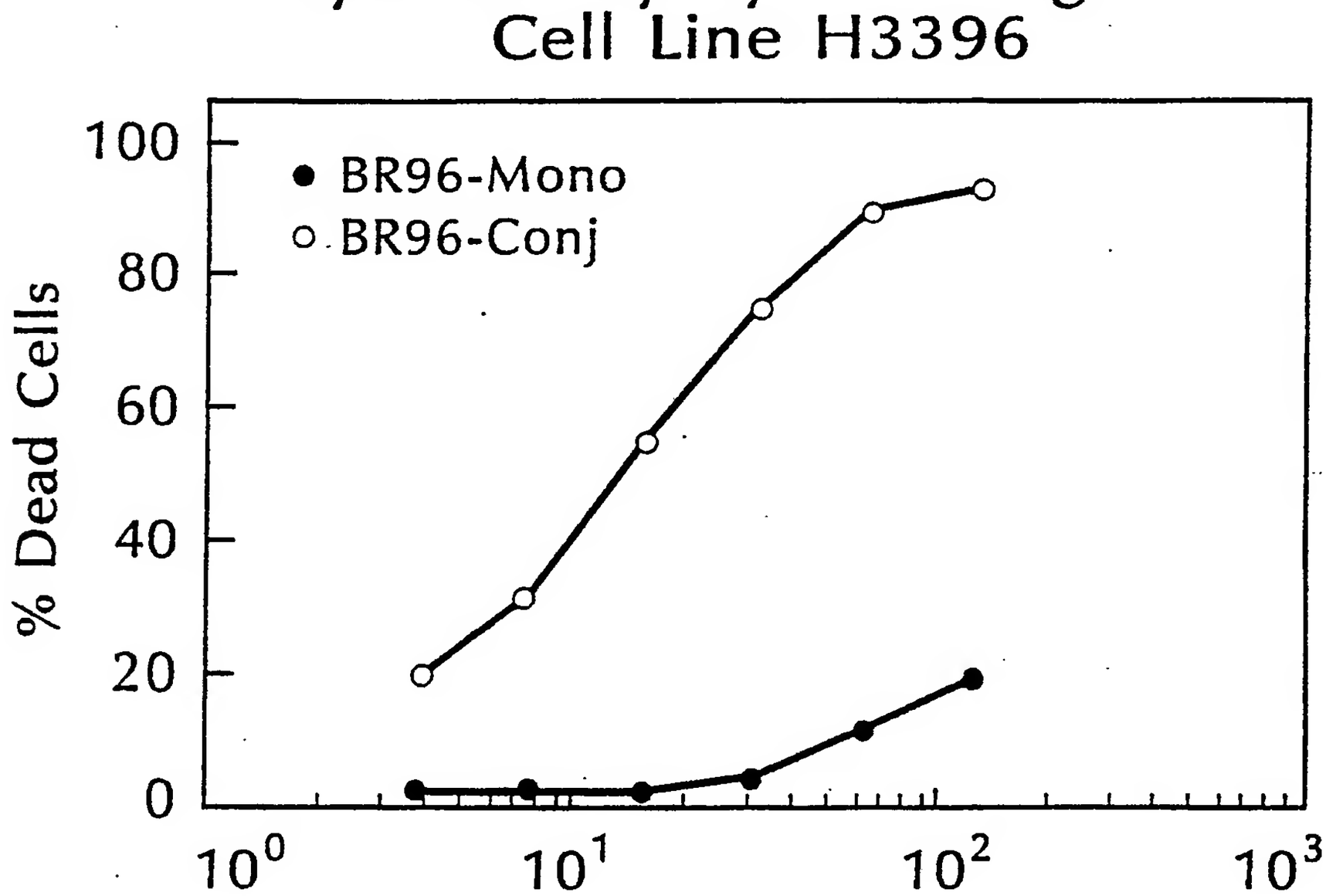

Cytotoxicity by BR96 Against
Cell Line H3396
100
80
60
40
20
0
% Dead Cells
BR96-Mono
BR96-Conj
10^0
10^1
10^2
10^3
Antibody Concentration (μg/ml)

Figure 11

Protection by Homoconjugate Against Group B Strep Infections

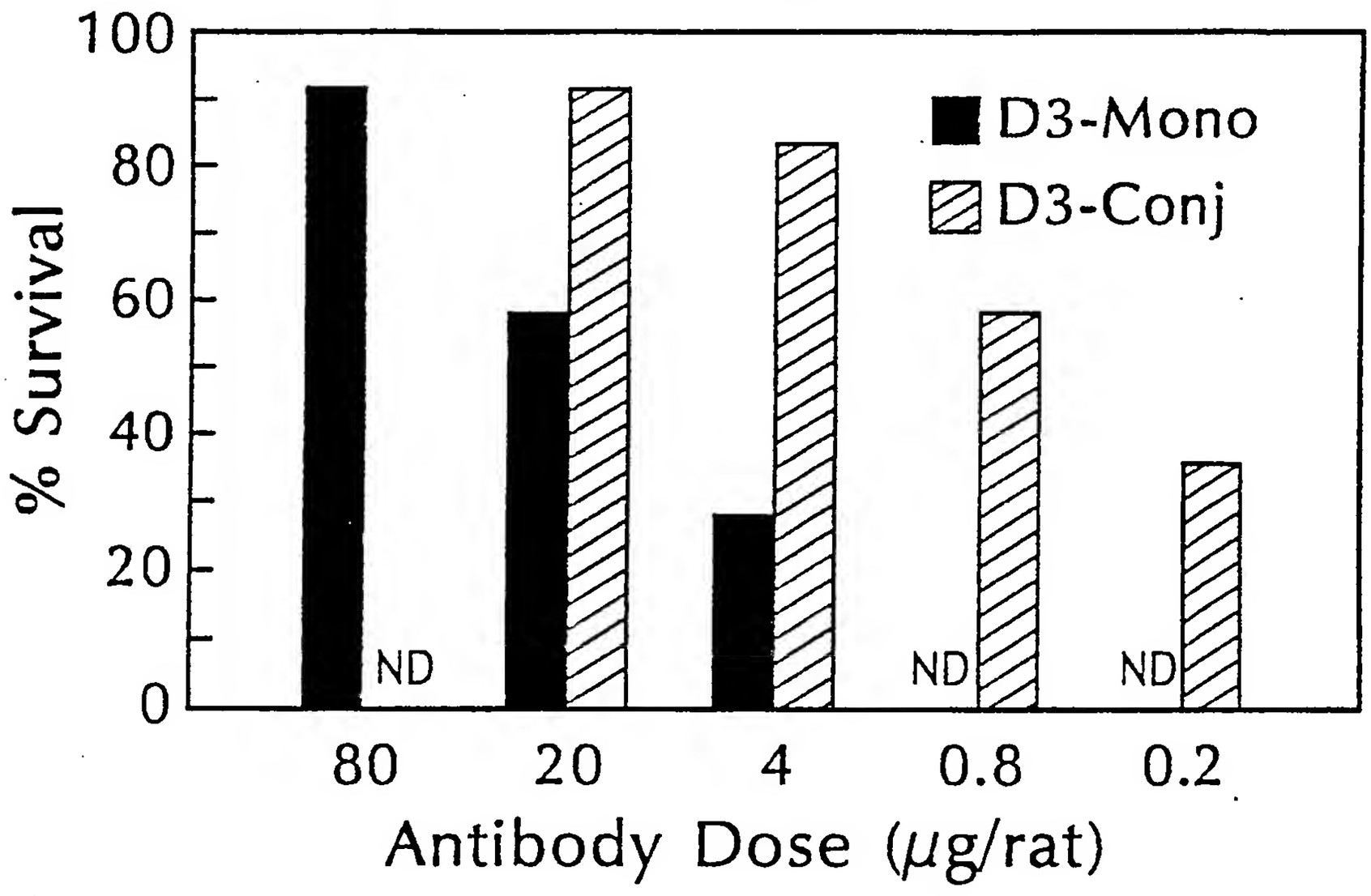


Figure 12

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06195

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 39/395

U.S. CL.: 424/85.8

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/85.8; 530/387; 435/240.27

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

DATABASES: **DIALOG** (Files 5, 155, 73, 72, 76, 159, 399, 440, 144, 34)
USPTO AUTOMATED PATENT SYSTEM (File USPAT; 1971-1990)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	J. EXP. MED., Volume 168, issued September 1988, **RAFF, H.V., ET AL.**, "Human Monoclonal Antibodies To Group B Streptococcus", pages 905-917, see the entire document.	1-22
Y	J. CHROMATOGRAPHY, Volume 489, Issued 1989, **BUSH, D.A. ET AL.**, "ISOELECTRIC FOCUSING OF CROSS-LINKED MONOCLONAL ANTIBODIES", pages 303-311, see the entire document.	1-14
Y	**V. GHETIE, ET AL.**, METHODS IN ENZYMOLOGY, Volume 92, issued 1983, pages 523-543, "Preparation and Applications of Multivalent Antibodies with Dual Specificity", see the entire document.	1-14
Y,P	SCIENCE, Volume 252, issued 03 May 1991, **SHUFORD, W. ET AL.**, "Effect of Light Chain Oligomerization and in vivo Efficacy", pages 724-727, see the entire document.	1-22

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

04 October 1991

Date of Mailing of this International Search Report

04 NOV 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

Susan L. Futrovsky (vsh)

Form PCT/ISA/210 (second sheet) (Rev.11-87)